EVALUATING THE EFFECT OF QUEBRACHO TANNIN SUPPLEMENTATION UPON RUMINANT PRODUCTION

A Dissertation

by

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ABSTRACT

Ruminant production is essential to meeting the high-quality protein requirements of an increasing global population. However, gaseous byproducts from ruminant production such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) can reduce energy efficiency and be detrimental to the environment. Increased societal awareness of food safety and environmental stewardship has driven the search for natural feed additives that can assist in mitigating greenhouse gases from animal agriculture. Condensed tannins are a diverse group of naturally occurring secondary metabolites that are potential alternative feed additives within ruminant nutrition due to improved protein efficiency and CH_4 mitigation. We investigated how quebracho tannin (QT) extract inclusion at differing rates, 0, 1.5, 3, and 4.5% DM, affected digestibility parameters, enteric gas production, energetic efficiency, nitrogen retention, and fecal gas flux in beef steers. The inclusion of QT above 1.5% decreased the digestibility of DM, organic matter, and nitrogen ($P < 0.01$) with variable responses for fiber digestibility across trials. Addition of QT altered excretion profiles with fecal N-to-total N excreted ratio and fecal N-to-urinary N ratio increasing with inclusion of QT, however, N retention was not different. Animals receiving QT had increased fecal energy ($P < 0.001$), resulting in decreased digestible energy ($P <0.01$). There were no differences in urinary energy, but the inclusion of QT reduced gas energy ($P < 0.01$). Metabolizable energy was not different across treatments with all inclusion levels maintaining a metabolizable energy-to-digestible energy ratio of 0.86 – 0.87. Heat energy decreased (*P*

= 0.01) with increased QT inclusion rate, but there was no difference in retained energy. For fecal gas flux trials, season and animal greatly impacted emissions, resulting in large variation between trials. The daily flux of CO_2 was influenced by soil moisture and temperature ($r = 0.34$; $P < 0.01$), whereas CH_4 and N_2O were associated with soil moisture. Large variation in animal response to QT resulted in discrepancies among trials. However, within certain environments QT supplementation could potentially improve animal performance and reduce fecal gas emissions. Future studies that encompass greater animal variability are required to determine the feasibility of QT utilization.

DEDICATION

I would like to dedicate this dissertation to my family, without everyone's support this would have never been possible, I love you all.

Mom, you have always believed in me and supported me no matter the circumstance, for that I want to say thank you. Love you forever.

Granny and Papa, without you two I would be lost. You both have done far more for me than I deserve, thank you for always supporting me. I love you both dearly.

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Contributors

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1. INTRODUCTION AND LITERATURE REVIEW

1.1. Animal and Environment Nexus

1.1.1. System Efficiency

The classical definition of sustainability is the use of a resource in a manner so that the resource is not depleted or permanently damaged; whereas natural resources are defined as materials and capacities supplied by nature. Sustainable intensification within agriculture requires meeting nutritional requirements of the world's exponentially growing population (United Nations, 2017) without negatively impacting the ecological system from which goods are obtained while using acceptable and profitable practices. For improvements in production practices to meet all requirements of sustainable intensification, a sound understanding of the system dynamics, thermodynamics, and their interactions within the system are required for determination of management process applicability and feasibility.

Ecological systems are comprised of numerous interconnected biotic and abiotic communities that influence energy flow- i.e. production - through positive and negative feedbacks that provide system stability (Holling 1973; Munang et al. 2011). Unfortunately, within ecological systems, vicious cycles that decrease system recovery and resiliency reinforce themselves through a feedback loop and typically occur at a faster rate than virtuous cycles. This implies that damaging the system will adversely impact production at a faster rate, whereas improvements to the system might not enhance production for an extended period. This delayed recovery is exemplified by

global population growth which has exhibited a decline in annual growth rates for almost 50 years, yet absolute annual growth has only recently been observed (FAO, 2017). Tedeschi et al. (2015) identified another all too real case of a vicious cycle that is currently in course. The authors illustrated that as the global population continues to rise an increase in food production is required; however, this increase in production will directly impact the environment by consuming non-renewable resources that contribute to global warming through emissions and decreasing water quality via superfluous nutrient application. This negative impact upon the environment subsequently leads to a harsher climate by depleting soil quality, increasing temperature, decreasing biodiversity, and loss of adapted animals and crops, offsetting previously improved productivity. Projections estimate that global food production must increase by 50% from 2012 levels to meet global demand in 2050 (FAO, 2017), so it is apparent that global system collapse is imminent rather than an unfathomable possibility. Therefore, the goal of agriculture should be to moderate the impact of this inevitable catastrophe by improving the efficiency of system processes through strategic management that allows progressive improvement without being inhibitory to the system, enabling a new, sustainable equilibrium to be set that would ameliorate even more catastrophic losses.

Energy is the currency of all defined systems and powers all processes within, whether large or small, biotic or abiotic. However, energy often goes unnoticed as it is either intangible or unrealized. The first law of thermodynamics states that energy can neither be created nor destroyed only transferred or changed from one form to another, whereas the second law of thermodynamics states that the energy available after a

chemical reaction is less than at the beginning of a reaction, or that energy conversion is not 100% efficient. This is commonly denoted as entropy, the degree of disorder in a system or energy unavailable for useful work. Additionally, reactions favor those that are thermodynamically favorable, spontaneous reactions, whereby the reaction proceeds in the forward direction. Spontaneous reactions will progress without the input of energy, with exception to the activation energy, and free energy of reactants is greater than those of products, whereas nonspontaneous reactions are the inverse. Determination of thermodynamic favorability is performed using Gibbs energy change (ΔG) with $-\Delta G$ indicating a forward reaction, $+\Delta G$ signify a reverse reaction, and $\Delta G = 0$ being equilibrium (Van Lingen et al., 2016). Those reactions or pathways within set parameters with a more negative ΔG will be thermodynamically favored, and therefore, utilized to a greater extent (Janssen, 2010). This plays a vital role in determining production efficiency and provides plausible routes of improving the efficiency of a system, with an applicable example being end-products of ruminal fermentation and the subsequent effect upon production and the environment (Kohn and Boston, 2000; Janssen, 2010; Van Lingen et al., 2016).

Biological processes are considered an open system whereby energy is exchanged with surroundings. Within these systems no process is isentropic; when energy is transferred or transformed, entropy is increasing to some degree (Scott, 2008). This includes highly ordered cellular processes since exergonic (catabolic) and endergonic (anabolic) reactions are taking place continuously. Unavailable energy from biological systems is commonly in the form of gas and heat as they are not greatly

utilized for work, in most instances, and therefore viewed as waste products of energy transformation. Greater heat energy increases the number of possible molecular states, further increasing entropy within the system. As a result, with progression through the food chain to higher trophic levels, there is decreased total free energy (energy transferred between trophic levels is approximated at 10%; Kozlovsky, 1968), increased net energetic waste products (heat and gas), and reduced efficiency of energy utilization. Although the concept of energy is abstract and energetic losses often seem negligible, such as in cellular processes, these inefficiencies have a large bearing on production efficiency. For example, improved nutrient efficiency for maintenance in comparison to that of growth can be partly attributed to cellular processes being nonspontaneous reactions (requires energy) that result in increased entropy (Turner and Taylor, 1983; Williams and Jenkins, 2003). As all macro-processes of systems are based upon unidirectional energy flow towards a more dispersed state, strategic energy improvements within segments of the system can enhance the energy efficiency of subsequent processes.

Since energy flow is unidirectional within the system, the energy efficiency of the system is improved by minimizing energy lost as heat and gas. Within biotic components, free energy conservation can be beneficial to production as the opportunity to harness free energy is increased. Within an organism, energy is first utilized for maintenance functions with the remaining energy being used for production processes; therefore, improving energy efficiency potentially provides greater free energy for production. Organisms largely benefit from energy conservation, but improved global

system efficiency is highly dependent upon the form in which energy is lost. As the earth maintains an energy balance, the sum of energy reaching earth from solar energy and energy produced from earth must be equivalent to energy leaving earth via radiation (absorbed + produced = dissipated). Otherwise the earth will warm. Although counterintuitive, energy lost from the animal production system as heat energy, and eventually to space, is favored over gas production due to the capacity for gases, such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), to entrap heat and alter climatic trends (Schneider, 1989). As the proportion of gas to heat energy increases the total heat leaving the system is decreased, negatively impacting the whole-system. Therefore, the determination of methods that can reduce heat and gas production is of great importance to both animal production and ecological systems.

1.1.2. Benefits and Challenges of Ruminant Production

The basis of ruminant production is the utilization of land areas deemed unfit for cultivation to produce human edible product (HEP). When ruminant sustenance is primarily from vegetative plants, the conversion efficiency of energy and protein can greatly exceed the nutritional value of what was consumed with energy and protein values increasing by factors of 3 and 6, respectively (CAST, 1999). The ability of ruminants to transform humanly inedible feedstuffs into HEP is possible due to the symbiotic relationship between the animal and microflora present within the reticulorumen. Anaerobic fermentation within the reticulo-rumen provides the animal with energy and protein in the form of volatile fatty acids (VFA) and microbial crude

protein (MCP), respectively. The type and amount of VFA and MCP provided to the animal vary greatly based upon the feedstuff consumed (Russell et al., 1992), thereby significantly influencing animal efficiency and emissions.

During the process of microbial fermentation, gaseous byproducts, such as ammonia (NH_3), hydrogen sulfide (H_2S), CO_2 and CH_4, are produced as waste products, but can also serve to maintain ruminal homeostasis. Although beneficial to animal wellbeing, byproducts of enteric fermentation reduce animal efficiency and are ultimately detrimental to air quality and augment global warming. In terms of global agricultural non-CO_2 emissions, enteric fermentation (~40%) and manure on pasture (~15%) represented 47 - 56% of total emissions in 2010 (Smith et al., 2014; Tubiello et al., 2014). Additionally, excessive N within excreta and subsequent conversion to specific compounds can negatively impact the environment by increasing fine particulate aerosols, leaching of nitrate into aquifers, runoff into surface waters leading to eutrophication, vegetational shifts due to increased N levels, soil acidification, and production of N_2O (Ndegwa et al., 2008). Ruminants have the capacity to upgrade feedstuffs to HEP; however, management strategies that enhance animal performance and reduce environmental ramifications are essential to improving system efficiency.

1.2. Metrics

1.2.1. Emission Metrics

A major source of energetic inefficiency and public criticism is greenhouse gas (GHG) production from ruminants and their impact upon natural resources and the

conservation thereof. Public criticism is warranted, as the agricultural sector accounted for 56% of global non-CO_2 emissions in 2005 (U. S. EPA, 2011), with emissions expected to increase 18 and 30% by the year 2030 and 2050, respectively (Tubiello et al., 2014). Emission trends and statuses are commonly compared using CO_2 equivalent (CO_2e) emissions, whereby gases are scaled to the reference gas CO_2. Gases are converted to CO_2e using global warming potentials (GWP) for individual gases, enabling equivalent comparison of radiative forcing for a given time horizon (Smith et al., 2014). Summation of CO_2e for all non-CO_2 gases from a source provides total CO_2e for comparison. Total CO_2e do not commonly encompass CO_2 as it is presumed that CO_2 will naturally cycle through environmental systems at differing rates, preventing determination of a definitive time horizon. When discussing emissions from agricultural systems, the primary GHG, and respective 100-year GWP are CO_2 (1), CH_4 (28), and N_2O (265) (IPCC, 2014; Tubiello et al., 2014). It is evident that CH_4 and N_2O can greatly impact global warming; however, even though CO_2 is utilized as the reference gas, it greatly contributes to global warming with an atmospheric lifetime up to several thousand years and therefore should not be discounted (Archer et al., 2009). Determination of suitable production methods that meet criteria of sustainable intensification, as set forth by Makkar (2013) and later expanded upon by Tedeschi et al. (2015), requires accurate estimates of emissions from differing production systems. Realization of emission inventories from primary emission components of differing systems can enable utilization of HEP-to-CO_2e ratio as a standard metric to assist in

promoting greater whole-system efficiency and possibly improving individual animal performance.

A major point of emphasis for the utilization of emission metrics, apart from determining livestock's direct contribution, is an inventory of soil carbon sinks within and among differing production systems. The global soil carbon pool is roughly 3 and 4.5 times the size of the atmospheric and biotic carbon pools, respectively, with approximately 60% present as soil organic carbon (SOC) (Lal, 2004a; Lal, 2004b; Morgan et al., 2010). This provides the opportunity for soil carbon sequestration to help offset anthropogenic emissions, at least for a period of time until the soil sink capacity is filled (Lal, 2004a). Although both components of the soil carbon pool, soil inorganic carbon (SIC) and SOC, play vital roles in carbon sequestration, SIC does not provide rapid sequestration because it is associated with the weathering of parent material and the formation of secondary carbonates which is a slow process (1 - 14 kg C ha^{-1} yr^{-1}), and requires specific soil and environmental conditions (Lal, 2008). In contrast, SOC is primarily a product of biotic residue decomposition, making it responsive to management practices and advantageous to carbon sequestration. Annual SOC sequestration under best management practices can range from 100 - 1000 kg C ha^{-1} yr^{-1} for croplands, 70 – 300 kg C ha^{-1} yr^{-1} on rangelands, and 300 – 1350 kg C ha^{-1} yr^{-1} in pastures with potential for SOC sequestration being greater for grasslands than that of croplands (Morgan et al., 2010; Lal et al., 2015). Excluding Greenland and Antarctica, grasslands encompass roughly 40% of the global land area with pasture and rangelands

accounting for 31 – 37% of lands within the USA (Follett and Reed, 2010; Morgan et al., 2010; Lal et al., 2015).

Since grasslands encompass a vast area and SOC is responsive to management practices, incremental management improvements that promote increased net primary production are promising for improvement of SOC sequestration (Conant et al., 2001). Utilization of grazing commonly imparts a trade-off between the economic return from live-weight gain and soil health due to the influence upon above- and belowground net primary production and associated factors (i.e., biodiversity, microbial activity, soil moisture and temperature, etc.) (Lal, 2004a; Mcsherry and Ritchie, 2013). However, the use of moderate grazing intensities has demonstrated average daily gains similar to those of higher stocking densities without compromising soil quality or forage persistence (Da Silva et al., 2014). A potential benefit of grazing for carbon sequestration is increased root-to-shoot ratio in response to defoliation; as root litter is of reduced quality relative to the shoot and directly enters the soil, it may represent a more reliable source of recalcitrant carbon (De Deyn et al., 2008; Pineiro et al., 2010). Unfortunately, the influence of grazing upon carbon dynamics is often confounded or misperceived as many carbon studies utilizing grazing are short-term, do not account for bulk density (Pineiro et al., 2010), or only measure shallow to intermediate soil profile depths (< 40 cm) (Mcsherry and Ritchie, 2013), leading to erroneous estimations of carbon balance and the contribution of belowground net primary production to the carbon pool. In the meta-analysis performed by McSherry et al. (2013) short term studies that sampled soils at 15 – 40 cm saw a negative effect of grazing whereas deeper depths (> 40 cm)

demonstrated positive effects. Therefore, a better understanding of the influence of grazing upon carbon dynamics utilizing diverse locales and forage species is a prerequisite to determining efficient management practices from an ecological and production perspective.

1.2.2. Animal Metrics

As discussed previously, improving energy efficiency within the animal increases the potentially available energy that can be used for production processes. Within beef cattle production, the animal is the final trophic level, not accounting for humans, and greatly influences system processes, making it pertinent to understand the mechanisms involved in improving energy efficiency. As the laws of thermodynamics and conservation of energy apply to all matter, in order for the oxidation of feedstuffs to yield energy for processes and storage within a living system a loss in the form of heat will occur (Van Soest, 1994). Therefore, net energy balance/equilibrium is the sum of all processes within the living system. Discussion of these processes is commonly performed on a macro-level with energy being fractionated into forms of energy loss and remaining energy for maintenance/production processes.

Energy flow within ruminants begins with the total energy of the diet or that which is consumed, termed gross energy (GE) for the diet or GE intake (GEI) respectively. Gross energy is the heat released upon complete oxidation of an organic substance and is related to the chemical composition of a feedstuff but it is not a determinant of energy available to the animal (NASEM, 2016). Digestible energy (DE)

is the GEI minus fecal energy (FE). Digestible energy is commonly termed apparent digestibility since it is not a good indicator of available energy, particularly in ruminants, as major energy losses via gas, heat, and urine, are not taken into account. The DE of a feedstuff can vary greatly as digestibility is dependent upon multiple factors including type of feed, stage of plant maturity, feed weathering, breed type, and the animal stage of production. Due to the high variability and unaccounted energy losses, inaccurate estimation of feedstuff digestibility is common for DE.

Metabolizable energy (ME) is an improvement upon DE as urinary and gaseous energy (UE & GASE) losses are accounted for; thus ME = GE - (FE + UE + GASE) or ME = DE - (UE + GASE). Although UE and GASE losses are included in ME, it is not a great advancement from DE due to ME and DE being highly correlated and reliable approximations of UE and GASE can be made from DE (NASEM, 2016). The majority of GASE is from microbial fermentation that also produces heat; however, heat production is not accounted for by ME. Traditionally, the ratio of ME:DE has been considered 0.82; nevertheless, ME:DE is highly variable with efficiencies of growing animals ranging from 0.82-0.93 (Vermorel and Bickel, 1980) and often exceeding 0.90 for feedlot diets (Hales et al., 2013, 2014, 2015, 2017). Using meta-analysis (n = 85), Galyean et al. (2016) demonstrated that ME and DE have a strong linear relationship; however, due to ME being dependent upon diet composition they suggested a fixed ME:DE ratio of 0.82 be utilized for low-quality diets (ME < 2 Mcal/kg), whereas application of the linear equation, ME = 0.96 × DE - 0.2999, may be more suitable for diets of increased quality (ME ≥ 2 Mcal/kg).

Metabolizable energy can only be further fractionated into either heat energy (HE) or retained energy (RE). Therefore, ME = RE + HE or RE = ME - HE. This relationship with RE and HE enables ME to be utilized within net energy (NE) systems. Net energy can be partitioned into NE for RE, milk energy, conceptus energy, and tissue energy, whereas HE is divided into HE required for maintenance, activity, and intake. Utilization of NE is advantageous due to energy values for different physiological functions being separated and NE requirements being independent of diet. Calculation of NE requires RE determination from at least two levels of intake energy (IE), illustrated as NE = ΔRE / ΔIE. The relationship between feed intake and RE is curvilinear, but Lofgreen and Garrett (1968) demonstrated that this did not differ from a linear relationship. Therefore, this method of NE determination assumes a linear relationship; whereby the intersection of the two lines is the point that RE=0, equal to maintenance. The HE when IE = 0, fasting heat production (FHP), corresponds to the NE required for maintenance (NE_m) resulting in no net gain or loss of energy from the animal and is determined as follows: NE_m = FHP / I_m whereby I_m is the amount of feed consumed at RE = 0. Similarly, NE retention (NE_r) is determined by: NE_r = RE / (I - I_m) where (I - I_m) corresponds to the level of feeding above maintenance.

Maintenance energy requirements account for regulation of body temperature, essential metabolic processes, and physical activity; however, the proportion of ME required for maintenance varies greatly depending upon age, body weight (BW), sex, breed, environment, physiological status, and the previous plane of nutrition (NASEM, 2016). The partial efficiency of energy utilization for maintenance (k_m) exceeds that for

growth (k_g), but the efficiency of ME utilization varies dependent upon diet and class of animal. Tedeschi et al. (2002) saw reduced k_m (63.7 vs. 69.9) and k_g (38.5 vs. 52.7) for bulls versus steers, respectively, when fed a high forage diet; although differences would likely remain, the number of bulls (n =31) within the study was greatly reduced in comparison to steers (n = 66) and could have potentially reduced accuracy of the efficiency estimations. In contrast, no difference was noted among F1 Nellore × Red Angus bulls, steers, and heifers for k_m (72.1, 70.6, and 71.3, respectively) or k_g (54.5, 47, and 54.3, respectively) when fed a high-quality diet (Chizzotti et al., 2007). Similar k_m was seen, 65 to 69%, when a finishing ration was offered to steers of diverse genotypes (Ferrell and Jenkins, 1998). On average, when not accounting for diet or sex/castrate status, the reported average k_m and k_g by Blaxter (1989) of 70% and 50%, respectively, appear to be similar to the meta-analysis of Nellore performed by Chizzotti et al. (2008) with k_m and k_g values of 67 and 44%, respectively. The difference in k_m and k_g is largely due to increased entropy in the form of GASE, HE, and UE from increased metabolic processes associated with the elevated intake (Turner and Taylor, 1983; Williams and Jenkins, 2003). However, many variables play a role in the efficiency of energy utilization for maintenance or production as it is largely a dynamic function of intake, the physical and chemical makeup of the feedstuff, digestive kinetics, and products of fermentation.

1.3. Animal Efficiency

1.3.1. Rumen Ecosystem

The rumen ecosystem is based upon a consortium of different microbial species that play a significant role in the degradation of feedstuffs that ultimately supply the host animal with protein and energy in the form of MCP and VFA. The overall value of the symbiotic relationship between rumen microflora and host animal is largely dependent upon the diet provided to the animal. Within forage-based systems the presence of microbes and associated enzymes is advantageous due to the capacity to digest feedstuffs, via fermentation, that is otherwise largely insoluble to mammalian enzymatic digestion as seen within monogastric animals. In contrast, when provided highly processed concentrate diets, the efficiency of fermentation decreases due to the efficiency of starch fermentation being only 70-75% of digestion and absorption within the small intestine (Harmon and Mcleod, 2001). Therefore, an understanding of the different primary microflora (bacteria, protozoa, and fungi) and their effect upon degradation of different dietary constituents enables a better understanding of ruminal dynamics and potential methods of improving efficiency.

Bacteria are the most prevalent microflora within the rumen, with up to 10^{10} cells g^{-1} of ruminal contents with the majority being gram-negative obligate anaerobes (Russell, 2002). Bacteria's role within the rumen ecosystem is rather diverse in comparison to other microflora, as some possess cellulolytic properties while others have amylolytic or proteolytic capabilities. As cross-feeding constitutes a major role, many bacterial species rely on the release of soluble sugars or byproducts by those

exhibiting amylolytic and cellulolytic properties. Therefore, no matter what substrate is introduced to the rumen, bacteria will commonly make up the vast majority of the microbes within the system. However, there are differences in the overall prevalence of bacteria for specific purposes, as cellulolytic bacteria are comprised of three major species, whereas the number of amylolytic bacteria exceeds five species (Nagaraja, 2016).

In contrast, protozoa are known for their large size and high motility and are largely anaerobic. They are comprised of two groups: flagellates and ciliated, with the former having insignificant effects on rumen processes and the latter making rather substantial contributions (only ciliates will be discussed). Protozoa are further grouped into holotrichs that have cilia covering the entire surface and entodiniomorphida that have cilia on the anterior portion. Holotrich ciliates commonly utilize soluble sugars, and their prevalence within the rumen is low with exception to post-feeding. In contrast, entodiniomorphs are predominant within the rumen and commonly ingest plant particles, structural CHO, and starch granules. All ciliates ingest bacteria as their major source of protein, thereby playing a pivotal role in the maintenance of a stable rumen environment when high concentrate substrates are fed by reducing the rate of starch fermentation either directly by engulfing starch or indirectly by controlling bacterial numbers (Russell, 2002; Nagaraja, 2016). Additionally, ciliated protozoa commonly support methanogens that serve as hydrogen (H_2) sinks that can inhibit fermentation by directly inhibiting the primary acetate forming pathway and release of H_2 from NADH (Hegarty and Gerdes, 1999).

As with protozoa, fungi are separated into two broad groups, yeast and mold, with the former having little to no influence on rumen processes. Molds are then split into aerobic and anaerobic categories with the anaerobic forms being of great importance to fermentation. The proliferation of fungi is typically increased with the presence of structural CHO due to increased prevalence of methanogens to utilize formate and H_2 byproducts from fungi (Nagaraja, 2016). This cross-feeding increases the thermodynamic favorability of fungi digestion processes with a resultant increase in ATP production. As fungi produce enzymes required for digestion of cellulose, hemicellulose, pectin, amylose, and protein, they are able to contribute greatly to digestion and fermentation of CHO. Additionally, they produce phenolic esterases that can break hemicellulose and lignin cross-bridges. The ability for fungi to produce rhizoidal protrusions enables penetration of plant tissues, increasing degradation of forages (Krause et al., 2003). This in combination with phenolic esterases is likely of great importance within roughage-based diets as they provide means of substrate colonization by hemicellulolytic and cellulolytic bacteria. As bacterial colonization of roughages is a primary constraint to substrate degradation, the presence of fungi indirectly promotes more efficient utilization of CHO. However, fungi exhibit reduced growth and degradation rates that affect their ability to persist due to being slower than the ruminal dilution rate (Krause et al., 2003).

1.3.1.1. Structural Carbohydrate Digestion

Although the evolution of ruminants revolved around the use of roughages as a feedstuff the number of bacteria capable of breaking down cellulose is meager as only three major species possess cellulases (Russell, 2002; Nagaraja, 2016). These species are *Ruminococcus albus*, *R. flavefaciens*, and *Fibrobacter succinogenes* with all three exhibiting slow growth and relying primarily on cellulose with only minor use of hemicelluloses and pectin (Russell et al., 1992; Nagaraja, 2016). Although these three are the primary cellulolytic bacteria, some non-cellulolytic bacteria have the ability to digest hemicellulose, *Prevotella* spp., *Butyrivibrio fibrosolvens*, and *Pseudobutyrivibrio xylanivorans* (Nagaraja, 2016). Pectin, in contrast to other substrate constituents, is a structural polysaccharide that is readily digested within the rumen by numerous bacterial populations. Unlike bacteria, all fungi have the capacity to digest cellulose, hemicellulose, and pectin. As noted previously, the presence of fungi likely catalyzes the colonization of structural CHO by bacterial populations. When a substrate is roughage-based, levels of soluble CHO are relatively low as structural CHO predominate. Reduced soluble CHO levels decrease the rate of fermentation and proliferation of bacteria; however, the fungi population commonly increases due to a greater number of methanogens. Therefore, this consortium of microbes that possess the ability to breakdown structural CHO is of great importance as they directly influence rumen kinetics and energy available for use by the animal, but it is evident that efficiency and rate of microbial digestion are dependent upon a highly dynamic system.

1.3.1.2. Non-structural Carbohydrate Digestion

The digestion of non-structural CHO (starch and sugar) is enzymatically more common and a much faster process than that of structural CHO. When a substrate consists of high levels of non-structural CHO, the increased rate of fermentation can lead to ruminal distress, such as acidosis and bloat, albeit dependent upon multiple factors such as intake, buffering capacity, and rates of acid utilization and absorption (Nagaraja and Titgemeyer, 2007). Starch is rapidly digested by amylolytic bacteria that have alpha-amylase and pullulanase enzymes for debranching amylose and amylopectin. Some examples of these amylolytic bacteria include *Ruminobacter amylophilus*, *Selenomonas ruminantium*, *Streptococcus bovis*, *Lactobacillus* spp., and *Bifidobacterium* spp. (Nagaraja and Titgemeyer, 2007; Nagaraja, 2016). Additionally, protozoa engulf starch granules, whereas fungi commonly contribute minimally to starch degradation. Reduction in fungi population is largely due to the rate of fermentation increasing the ruminal concentration of CO_2 and H_2, providing a surplus of precursors for methanogens. As a result, substrate degradation is less thermodynamically favorable since H_2 and formate, which is rapidly converted to CO_2 and H_2, are the primary byproducts of fungi metabolism (Hegarty and Gerdes, 1999; Nagaraja, 2016). Monosaccharide and disaccharide byproducts of starch hydrolysis are utilized by sugar fermenting bacteria such as *Streptococcus* spp., *Bifidobacterium* spp., *Lactobacillus* spp., and *Treponema* spp. Of these, *S. bovis* and *Lactobacillus* spp. are especially important due to their increased rate of proliferation and propensity to produce lactic acid. Lactate-utilizing bacteria, *S. ruminantium* ssp. *lactilytica* and *Megasphaera elsdenii*, are of equal

importance as they metabolize lactate to acetate, propionate, and butyrate, helping to reduce the accumulation of lactate that can lead to lactic acidosis (Russell, 2002; Nagaraja and Titgemeyer, 2007). Protozoa also play an integral role in reducing the incidence of acidosis through engulfing starch granules and reducing the proliferation rate of bacteria (Nagaraja and Titgemeyer, 2007). The digestion of non-structural CHO is a much more volatile process than structural CHO digestion, with feedback mechanisms in place to help maintain a stable environment and prevent rumen distress.

1.3.1.3. Protein Digestion

As most of the CHO fermenting bacteria also have proteolytic abilities, the rumen has very little free amino acids due to their rapid fermentation. This is commonly performed via deamination that almost all proteolytic bacteria are involved in. There is also a group of bacteria referred to as 'hyper ammonia producers' that exclusively hydrolyze peptides and deaminate amino acids (Nagaraja, 2016). Ammonia is a byproduct of N metabolism that can be utilized by most ruminal bacteria as a N source with microorganisms that ferment non-structural CHO able to utilize peptides, amino acids, and NH_3, whereas those that ferment structural CHO can only use NH_3 (Russell et al., 1992). Even though NH_3 is highly utilized by microorganisms, in many instances, protein lost as NH_3 exceeds 25% (Russell et al., 1992). Overproduction of NH_3 results in decreased animal efficiency and reduced environmental quality (Ndegwa et al., 2008). Therefore, it is important that excessive levels of protein are not provided to the animal

or that acceptable levels are ruminally protected so that energetic waste via N excretion within the urine is reduced.

1.3.2. Determinants of Ruminant Efficiency

From both an animal performance and environmental emissions standpoint, the least efficient point within the production chain is the cow-calf sector. This is largely due to diets being almost exclusively forage with negligible concentrate supplementation and animals transitioning through multiple production stages (gestation, lactation, and growth). Although roughage-based diets vary greatly among and within locales, collectively these diets are known to have lower efficiency when compared to concentrate diets. The underlying reason for this decreased efficiency is due to a culmination of greater energetic costs of eating and ruminating, increased metabolic activity of visceral organs (VO) and gastrointestinal (GI) tract, elevated acetate: propionate (A:P) ratio, and increased GASE. However, these assumptions may be misconstrued as research has continually demonstrated conflicting results. The primary factors, although heavily disputed, remain:

1.) Elevated A:P (exceeding 3.5:1 mol absorbed) reduces glucose or glucose precursors (propionic acid, amino acids, and glycerol) required for efficient use of acetate for long-chain fatty acid production, resulting in greater energy losses due to heat-generating substrate cycles (futile cycling) (van Houtert, 1993).

2.) Time spent eating and ruminating, not dry matter consumed (Adam et al., 1984), has been emphasized as major sources of energy loss (Orskov and MacLeod,

1990). This was further validated when it was demonstrated that there was no difference in heat production between concentrates and roughages when roughages were ground and pelleted (Agricultural Research Council, 1980).

3.) Level of intake has shown to increase the relative proportion of VO to body mass (Burrin et al., 1990) while increased metabolic activity (O_2 consumption) of visceral tissues has been demonstrated in diets with increased roughage (Reynolds et al., 1991), greatly influencing total heat production.

The theory of futile cycling was substantiated by Cronje et al. (1991) who demonstrated that lack of glucose and glucose precursors did not impact acetate flux rate, but inhibited acetate clearance rate. However, Orskov and MacLeod (1990) disputed that the cost of eating and ruminating were the primary reasons for lower energetic efficiency of roughage diets as there were not major energetic differences in utilization of acetic and propionic acid with only minor differences in energetic efficiency being due to GASE. The reviews by Orskov and MacLeod (1990) and van Houtert (1993) only briefly discussed, if at all, the influence of diet composition upon VO mass and GI tract attributes; however, the effect of diet composition upon VO and the lower GI tract likely has the greatest influence upon energetic efficiency. This is due to increased levels of dietary bulk being associated with greater VO and GI weight, size, and metabolic rate as a proportion of available energy for peripheral tissues, although dependent upon roughage quality (Koong et al., 1985; Rompala et al., 1988; Burrin et al., 1990; Reynolds et al., 1991). Therefore, as stated previously, efficiency is ultimately dependent upon a multitude of factors to varying degrees. Unfortunately, this indicates

that means of improving energy utilization of roughages are sparse as the processing of feedstuffs and decreasing dietary bulk, from an economical and practical stance, are largely impossible in grazing situations. However, improved efficiency is feasible through rumen modulation, via feed additives, to alter VFA profiles, promote nutrient absorption within the small intestine (SI), and reduce gaseous emissions for improved utilization of nutrients for maintenance and growth processes.

1.3.3. Feed Grade Antimicrobials

Historically, antimicrobials, have been utilized within feed and water to decrease animal morbidity and alter rumen dynamics, promoting growth efficiency. Feed grade antibiotics utilize bacteriostatic or bactericidal modes of action that affect either cell membrane permeability or protein synthesis of bacteria (Madigan et al., 1997; Brown et al., 2017), largely gram-positive, altering the populations and productivity of the rumen. Increased efficiency is provided by reducing deamination and NH_3 production, decreasing A:P, temporarily diminishing CH_4, and increasing DE, ultimately improving feed conversion (Bergen and Bates, 1984; Spears, 1990; Yang and Russell, 1993; Guan et al., 2006). A survey of consulting feedlot veterinarians revealed that 73.9% recommended the use of feed grade antibiotics for high-risk cattle, whereas 30.4% endorsed utilization in low-risk cattle (Terrell et al., 2011). This is supported by the US Food and Drug Administration (2015) summary report that indicated that most medically important antibiotics were delivered via feed while only 3.6% were administered by injection. Indiscriminate, sub-therapeutic use of antibiotics has led to reports of

microbial adaptation (Guan et al., 2006) while the rumen has been cited as a repository for resistance genes (Hitch et al., 2018) that could potentially pose a threat to human and animal populations. These potential threats prompted the FDA to enact the Veterinary Feed Directive Final Rule in December of 2016 that requires veterinary oversight of antibiotics deemed medically important. This ruling made it apparent that antibiotic use will begin to be phased out, or at least greatly reduced, within the U.S.A., as was done within the European Union. Therefore, it is of great importance that alternative methods of modulating rumen function for maintenance of current production efficiency be determined.

1.4. Condensed Tannins as an Alternative Feed Additive

Decreased efficacy and acceptability of feed-grade antibiotics requires that effective and publicly accepted feed additives be utilized within production. A possible alternative to antibiotics is a group of compounds termed condensed tannins (CT). Condensed tannins are a diverse group of naturally occurring secondary metabolites that are produced by plants as a means of coping with stress. They are formed primarily as oligomeric and polymeric flavan-3-ols connected by 4-6 and 4-8 linkages (Dixon et al., 2005). The presence of hydroxyl groups provides varying levels of reactivity via hydrogen bonding and hydrophobic interactions when in proximity to compounds such as proteins, carbohydrates, microbes, and enzymes (Haslam, 1989). Biological activity, the capacity of a substance to alter one or more chemical or physiological functions of a cell, tissue, organ, or organism, is commonly used to describe the propensity for a

specific CT to bind substrate. The extent of biological activity exhibited by polyphenols is a function of the chemical structure, degree of polymerization, molecular weight, stereochemistry, hydroxylation, monomeric sub-units, and pH, as well as the structure of the compound being complexed (Smith et al., 2005). The vast diversity and associated binding capability of CT makes them a prospect for simultaneously improving animal performance and mitigating environmental pollutants within a multitude of production scenarios.

The utilization of CT within animal agriculture, more specifically, ruminant production, has been researched heavily for more than a quarter of a century. However, the results of these many research ventures have been largely contradictory due to lack of suitable analytical techniques that enable discriminate use of CT for specific purposes (Mueller-harvey, 2006), leading to negligible use of CT in commercial animal production, with exception to minor supplementation in small ruminant production and grazing of CT containing forages. From Waghorn and McNabb (2003), the major demonstrated benefits and detriments of CT use within ruminant production are as follows:

Benefits

1. Improved weight gain
2. Improved reproductive efficiency
3. Increased milk production
4. Greater protein concentration in milk
5. Reduction of gastrointestinal nematodes

6. Increased tolerance to gastrointestinal nematodes
7. Improved antioxidant status
8. Prevention of bloat
9. Decreased CH_4 production

Detriments

1. Decreased voluntary feed intake (VFI)
2. Decreased nutritive value of other dietary constituents (CHO, microbial crude protein (MCP))
3. Reduced weight gain
4. Reduced overall performance, death in certain scenarios
5. Large variability among and within CT sources

The beneficial aspects presented are largely an indirect result of improved efficiency primarily by increasing substrate utilization efficiency and reducing heat production through the use of more efficient system processes. Increasing digestion and absorption of nutrients within the SI improves the efficiency of substrate utilization (usable nutrient/heat produced) by reducing gas production and substrate intermediates.

1.4.1. Nitrogen

The most promising and highly researched process by which CT can potentially enhance ruminant production is the improvement of N use efficiency. Condensed tannins can provide ruminal protection of protein, ruminal undegraded protein (RUP), by direct

complexation of the substrate and proteolytic enzymes or indirectly impacting proteolytic activity (Waghorn et al., 1987; Waghorn et al., 1994). Potential disassociation from the protein within the abomasum allows amino acid absorption within the SI (Barry and Manley, 1984; Barry et al., 1986; Waghorn et al., 1987). Reduction of N degradation by rumen microorganisms can decrease excess NH_3 production that must then be processed by the liver and kidneys for excretion via urine (Patra and Saxena, 2011). Processing and excretion of excess N as urea presents a source of energy loss within the animal and poses a threat to the environment. Supplementation of quebracho (*Schinopsis balansae*) CT at 3% (DM) decreased both ruminal NH_3 and milk urea nitrogen in dairy cows (Dschaak et al., 2011). A reduction in ruminal NH_3 was also seen with supplementation of *Acacia mearnsii* at 0.9, 1.8, and 2.7% (DM) to a high-roughage (55:45 Forage-to-Concentrate ratio) dairy ration (Orlandi et al., 2015). In most studies involving CT, a shift in the route of N excretion from the urine to feces was observed due to reduced ruminal proteolysis (Barry et al., 1986; Waghorn et al., 1987; Ahnert et al., 2015; Orlandi et al., 2015). Decreased substrate availability and direct or indirect inhibition of rumen microbes have the potential to negatively impact MCP production. Although highly generalized, at rates ≤ 3% CT (DM) MCP outflow from the rumen is not impacted, with an increase in MCP outflow being noted in some cases (Bhatta et al., 2000; McNeill et al., 2000; Min et al., 2003; Al-Dobaib, 2009). Supplementation of quebracho CT at 0, 1, 2, 3, and 4% (DM) within a high-roughage diet did not result in a change in total purine derivatives, MCP, or efficiency of microbial protein synthesis (Piñeiro-Vázquez et al., 2017). Mezzomo et al. (2011) noted that when

quebracho CT was supplemented at 0.4% (DM) to a high-concentrate diet there was no change in efficiency of microbial protein synthesis but an increase in metabolizable protein (+ 24%) and RUP that reached the abomasum (CT: 406 g/d vs. CON: 302 g/d). Thus, in terms of absolute protein values, CT enabled greater flow to the SI. Similar results were seen with *A. mearnsii* CT as no change in the efficiency of microbial protein synthesis or MCP within duodenal flow was seen, but RUP and total N reaching the duodenum, N retention, and efficiency of N utilization increased at all levels of CT inclusion (Orlandi et al., 2015). Increasing total protein that reaches the SI is highly beneficial as it provides amino acids that can potentially be used for tissue maintenance or growth and reduces energy losses, enabling increased growth rates in growing animals. Additionally, in diets that result in lower levels of propionic acid, increased amino acid levels can provide glucose precursors that allow efficient utilization of acetate for long-chain fatty acid production that otherwise would result in large energy losses due to heat-generating substrate cycles (van Houtert, 1993). Dschaak et al. (2011) reported a slight reduction in molar proportions of VFA and a lowered A:P ratio when quebracho CT was added within high-forage diets but not in the low-forage group. This demonstrates the possibility of increasing both propionic acid and amino acid provision within high-roughage diets. Whereas, during lactation increased amino acid absorption provides supplemental glucose precursors that enables the preservation of skeletal muscle from catabolism in times of low energy balance and can serve as a means of increasing milk production and quality through increased provision of glucose and amino acids (Herdt, 2000). Woodward et al. (2000) saw increased milk yield, protein,

and efficiency in dairy cattle when provided *Lotus corniculatus* versus *L. corniculatus* with PEG, ryegrass (*Lolium* L.), and ryegrass with PEG.

1.4.2. Digestibility

A major point of scrutiny is that CT may improve N efficiency but decrease digestibility of CHO in the process. High levels of unbound CT, common if dietary protein is low, can cause depressed CHO degradation through direct and/or indirect inhibition of microorganisms and enzymes (Barry and Manley, 1984; Patra and Saxena, 2009). The uppermost level of supplementation prior to affecting fibrous CHO digestion appears to be dependent upon the CT type. Provision of *L. corniculatus* at 2% CT (DM) and *A.mearnsii* up to 2.7% (DM) did not affect digestion (Waghorn et al., 1987; Orlandi et al., 2015), but supplementation of quebracho above 1.5% CT (DM) decreased digestibility of DM (DMD) and organic matter (OMD) in sheep and cattle (Al-Dobaib, 2009; Piñeiro-Vázquez et al., 2017). However, this response is variable and is likely dependent upon the basal diet. Supplementation of quebracho CT at 3% (DM) did not reduce DMD, neutral detergent fiber digestibility (NDFD), or acid detergent fiber digestibility (ADFD) in high (59:41 Forage-to-Concentrate ratio) or low (41:59 Forage-to-Concentrate ratio) forage diets consisting of alfalfa hay and corn silage (Dschaak et al., 2011). This is consistent with results of Beauchemin et al. (2007), where DMD, NDFD, and ADFD of a 70% silage diet was not impacted by quebracho inclusion at 1 and 2% (DM). When quebracho was provided at ≤ 1% (DM) within concentrate diets, DMD, OMD, NDFD, and ADFD were not affected (Benchaar et al., 2008; Mezzomo et

al., 2011; Ebert et al., 2017). Even though rumen degradation is often reduced, apparent digestibility may not be affected by CT, as *L. pedunculatus* exceeding 9.5% CT (DM) greatly reduced ruminal degradation of readily fermentable CHO but this was counteracted to an extent via post-ruminal digestion (Barry and Manley, 1984; Barry et al., 1986). However, this too varies as apparent total tract starch digestibility was reduced when quebracho CT was provided at 1% (DM) within a finishing diet, yet starch and non-fibrous CHO digestibility were numerically greater when quebracho provision was approximately 0.5% (DM) (Mezzomo et al., 2011; Ebert et al., 2017). Increasing post-ruminal digestion of non-fibrous CHO would be of energetic merit as it would allow direct glucose absorption within the SI while decreasing total heat and gas production. In contrast, decreased digestibility of fibrous CHO can cause shifts in A:P by decreasing the molar proportion of acetate (Carulla et al., 2005; Beauchemin et al., 2007). Reduction in A:P below 3.5:1 mol helps ensure adequate glucose precursors for efficient production of long-chain fatty acids from acetate (van Houtert, 1993). Provision of quebracho slightly reduced the total VFA and decreased the A:P ratio within high-forage diet but no effect upon digestibility was seen in the low-forage diet (Dschaak et al., 2011). In contrast, decreased A:P ratio was reported in high versus low CT *Sorghum bicolor* silage with a tendency for decreased NDFD and ADFD in the high CT silage (de Oliveira et al., 2007). Therefore, alteration of total VFA and VFA profiles appears to be due to both direct and indirect inhibition of microbes. In either scenario, caution must be taken. Decreased digestibility of structural CHO could be detrimental to production within roughage-based diets as diminished digestibility will ultimately limit MCP

production and passage rate. Consequently, intake can be reduced to the extent that nutrient requirements are not met.

1.4.3. Environmental Impact

The use of CT offers the unique opportunity to simultaneously improve animal production and reduce the environmental impact of animal agriculture. As stated previously, CT commonly shifts the route of N excretion from the urine to the feces (Barry et al., 1986; Waghorn et al., 1987; Ahnert et al., 2015; Orlandi et al., 2015; Ebert et al., 2017). From an environmental perspective, the primary benefit of shifting the route of excretion to the feces is a decrease in volatile N. Increased levels of N within excreta, most especially urine, can negatively impact the environment as discussed previously. The ability of CT to decrease NH_3 production and promote N flow to SI increases N excretion within the feces, providing two significant services. First, it reduces total urea excreted within the urine. Urea is rapidly converted to NH_3, a volatile atmospheric pollutant, or nitrate that can greatly diminish water quality through leaching and/or be converted by soil microbiota to N_2O (Eckard et al., 2010). Secondly, the CT-protein complex within fecal material decreases the rate of mineralization and denitrification due to microbial inhibition (Kraus et al., 2004; Powell et al., 2009; Eckard et al., 2010). Reducing mineralization rates of organic compounds to inorganic compounds, allows accumulation of organic matter that can provide a slow release of nutrients, enables greater plant uptake efficiency, and reduce nutrient loss (Farrell et al., 2014).

In addition to reducing N waste, CT have also exhibited the ability to mitigate enteric CH_4, although this appears to vary based upon the type of CT and substrate. Within forage-based diets *A. mearnsii* at 0.9 and 1.8% (DM) and quebracho at 2% (DM) reduced CH_4 production of cattle 15, 29, and 31%, respectively (Grainger et al., 2009; Piñeiro-Vázquez et al., 2017). Whereas when quebracho and sorghum CT were incorporated within silages, there were no effects upon CH_4 emissions of cattle (Beauchemin et al., 2007; de Oliveira et al., 2007). Similarly, when quebracho CT was included at 0.5% and 1% (DM) to a finishing diet, there was no change in CH_4 production relative to the control diet (Ebert et al., 2017). Although total daily CH_4 production of dairy cattle consuming ryegrass or *L. corniculatus* silages did not differ, CH_4/kg DMI and CH_4/kg milk solid yield were reduced in the *L. corniculatus* treatment (Woodward et al., 2001). Reduction of CH_4 is thought to occur through inhibition of ciliated protozoa populations, resultantly decreasing protozoal-associated methanogenesis. Sheep fed *A. mearnsii* at 2.5% (DM) demonstrated reduced CH_4 per unit of DMI and GEI. Although total ciliated protozoa and entodiniomorphs did not differ, holotrichs were reduced by more than 50% (Carulla et al., 2005). When QT was provided at 0.64% (DM) Benchaar et al. (2008) did not see a statistical difference in total protozoa or distribution by genera in dairy cattle. As CT can impact CHO digestibility, decreased CH_4 at times can be attributed to decreased fiber degradation in forage-based diets; however, this may actually increase the amount of CH_4 per unit of substrate digested. Therefore, caution is required when deciphering the true impact of CT upon energy efficiency.

Although CT supplementation could potentially serve as a method of improving nutritional efficiency, nutritional effects are often varied. In contrast, beneficial impacts upon the environment appear to be more predictable, to an extent. Therefore, strategic utilization of CT at rates that maintain or provide an opportunity to improve nutritional status while reducing environmental impacts may be most beneficial from a whole-system perspective. However, utilization of CT as a means of maintaining animal production while improving environmental stewardship requires determination of effect upon nutrient allocation to growth processes and associated impact upon enteric and excreta associated greenhouse gases.

1.5. References

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2. COMPARISON OF IN SITU TECHNIQUES FOR DETERMINATION OF INDIGESTIBLE COMPONENTS IN THE FEED AND FECES OF CATTLE RECEIVING SUPPLEMENTAL CONDENSED TANNINS

2.1. Overview

Reliable assessments of indigestible dietary components are required for utilization of internal markers to estimate dietary digestibility and digestible energy in ruminant animals. However, the lack of a standardized methodology can result in erroneous estimations with inconsistent variation across trials and among studies. Previous research has detailed suitable bag porosity and sample size (SS) with the assumption that the 288 h incubation length (IL) yields truly indigestible components. Recent research efforts have primarily investigated the variation that exists among feedstuffs, primarily forages, but most failed to account for possible effects of secondary compounds. In this study, the following factors were further investigated: bag type (BT; 10 and 25 μm), SS (20 and 40 mg/cm^2), and IL (288 and 576 h) upon in situ indigestible dry matter (iDM) and neutral detergent fiber (iNDF) of feed and feces residues, as well as resultant DM and NDF digestibilities of two similar concentrate diets, one of which contained supplemental condensed tannins (CT). There were no three-way interactions ($P > 0.05$) but all two-way interactions were present for iDM and iNDF residues with BT × SS influencing the control feed ($P < 0.01$), SS × IL impacting feed containing CT ($P < 0.01$), and BT × IL affecting both feedstuffs ($P \leq 0.01$). However, only BT × SS affected digestibilities in which DM and NDF digestibilities of the control diet were greater for

the ten μm BT and the 20 mg/cm^2 SS combination (P = 0.01 and 0.03, respectively). Values of iDM were largely influenced by contamination that varied greatly based on intrinsic factors associated with the bag and incubation duration. The presence of CT influenced iDM and iNDF to varying degrees due to possible trapping of CT-substrate complexes. Use of 25-μm bags resulted in lower fecal recoveries relative to the 10-μm (P < 0.01). Moreover, it is probable that the larger SS impacted microbial and enzymatic activity based upon the relative increase of iDM and iNDF. Our results suggest that 288 h incubations may not represent truly indigestible constituents when utilizing 20 mg/cm^2, as 576 h incubations exhibited lower residues and increased digestibility values. When investigating the effect of sample size required to attain 90% power, based upon our data when utilizing two incubation animals a sample size exceeding the triplicate and quadruplicate replications commonly utilized in experimentation is required.

2.2. Introduction

The proportion of neutral detergent fiber (NDF) within a feedstuff greatly influences animal performance by affecting organic matter digestibility and total intake, as well as serving as an important source of metabolizable energy (Harper and McNeill, 2015). However, NDF content is a nutritionally complicated concept because it is a heterogeneous entity that can be fractionated into potentially digestible (pdNDF) and indigestible (iNDF) fractions (Vieira et al., 2008). Though not chemically defined, iNDF describes the innate properties of the cell wall and serves as an ideal nutritional entity because its digestibility is zero (Mertens, 1993; Van Soest, 1994).

Indigestible NDF plays an integral role in the determination of pdNDF within nutritional models and serves as an internal marker for rumen kinetics and digestibility estimations (Sampaio et al., 2011; Palmonari et al., 2016). The accuracy of iNDF as an internal marker is dependent upon the incubation technique utilized, with bag type (BT) and incubation length (IL) serving as potential sources of error (Nocek, 1988; van der Koelen et al., 1992). Currently suggested methodology is the use of 288 h incubations and bags of 6 – 12 μm porosity (Krizsan and Huhtanen, 2013; Krizsan et al., 2015). Previous research investigated the use of commercially available F57 bags (25 μm; ANKOM Technology, Macedon, NY) for determination of indigestible feed residues with positive results (Casali et al., 2009; Valente et al., 2011), but particle loss due to bag porosity is a potential source of error when incubating heterogeneous particle sizes for a long period (Huhtanen et al., 1994). A recent commercial bag, F58 (10 μm; ANKOM Technology, Macedon, NY), provides the possibility to reduce bag variability and improve estimations of indigestible components when using heterogenous particle sizes. The objective of this study was to evaluate the effect of BT, sample size (SS), and IL upon indigestible dry matter (iDM) and iNDF.

2.3. Materials and Methods

The animals used in this experiment were registered and cared for according to guidelines approved by the Institutional Animal Care and Use Committee (AUP's 2016-0362 and 2017-0306) of Texas A&M University.

2.3.1. Sample Collections

Feed, orts, and fecal samples utilized for iNDF analyses were collected during a calorimetry trial performed in fall 2016. The calorimetry trial implemented a Latin rectangle design with four periods, four treatments, and eight English crossbred steers (320 ± 21 kg). Ingredient composition of the total mixed ration and chemical composition for control (CON) and 3% quebracho (*Schinopsis balansae*) condensed tannin (CT) dietary treatments are listed in Table A-1.

Upon removal from respiration chambers, fresh feces were collected directly from the anus and a subsample of total orts was collected. and stored at -20° C. Representative samples of each diet were made from four samplings during the last 8 d of each period. All samples were stored at -20° C prior to being dried at 55° C for 72 h using a forced air oven and ground to pass through a 2.0-mm screen (Wiley mill, Thomas Scientific, Swedesboro, NJ). A subset of each representative sample was shipped to Cumberland Valley Analytical Services (Waynesboro, PA) for chemical analysis of DM (Goering and Van Soest, 1970), NDF (Van Soest et al., 1991), ADF (Method# 973.18) (AOAC, 2000), CP (Method# 990.03) (AOAC, 2000; Leco FP-528 Nitrogen Combustion Analyzer, Leco Corporation, St. Joseph, MO), soluble CP (Krishnamoorthy et al., 1982), a complete mineral panel (Method# 985.01) (AOAC, 2000; Perkin Elmer 5300 DV ICP, Perkin Elmer, Shelton, CT), and calculation of non-fiber carbohydrates (NFC), total digestible nutrients, and net energy.

2.3.2. Experimental Design

A 2 × 2 × 2 factorial design was used to investigate the effect of BT, SS, and IL upon iDM, iNDF, and subsequent digestibility estimates for CON and CT diets. The determination of indigestible components was performed using F57 or F58 bags (ANKOM Technology, Macedon, NY), 25- and 10-μm porosity respectively, filled with a SS-to-surface area ratio of 20 or 40 mg DM/cm^2 (20 and 40 mg), and incubated for 288 or 576 h in the rumen of four ruminally cannulated English-cross steers (603 ± 15 kg). Feed, fecal, and ort samples for each animal, except two due to diet refusals, (n = 6) within diet (n = 2) were replicated 16 times for each incubation treatment combination. Sample bags were placed within 36 × 42 cm polyester bags, four polyester incubation bags per cannulated animal. During preliminary trials, it was noted that F58 bags sequestered fibrous particles on the outside of the bags; therefore, polyester incubation bags of 50 μm porosity were utilized rather than commercial laundry bags. A preliminary comparison of 50 μm incubation bags with commercial laundry bags was performed to ensure bag porosity did not affect iDM and iNDF residues (P= 0.57 and 0.2, respectively, data not shown). Timed placement methodology was utilized to ease bag removal and reduce labor. Cannulated animals were kept in a dry-lot with bermudagrass (*Cynodon dactylon*) hay provided ad libitum and supplemented every other day with 250 g of dried distillers grains. Upon removal from the rumen, all bags were immediately quenched in ice water followed by rinsing in a household washing machine using cold water and the rinse portion of the delicate wash cycle (Krizsan and Huhtanen, 2013).

All bags were dried at 55° C for 72 h using a forced-air oven, placed into desiccators and weighed to obtain iDM. Determination of iNDF was performed using the ANKOM 200 fiber analyzer (ANKOM Technology, Macedon, NY) and washed according to Van Soest et al. (1991) using a detergent-to-sample ratio of 100 mL/g DM and the addition of 4 mL of heat-stable amylase with sodium sulfite being omitted. Bags were then rinsed in hot water followed by soaking in acetone prior to drying at 55° C for 48 h. Values of iDM and iNDF were ash inclusive but blank bag corrected for BT × IL. Indigestible DM and NDF were calculated as a proportion of DM using the following equations:

[1] $\text{iDM, \%} = (W3-(W1\times C1))/W2\times 100$

[2] $\text{iNDF, \%DM} = (W4-(W1\times C2))/W2\times 100$

where W1 is the initial bag weight (g), W2 is the initial SS (g), W3 is the dried weight of bag containing sample residue following water rinse (g), W4 is the dried weight of bag containing sample residue following washing with ND (g), C1 is the blank bag correction factor following water rinse, and C2 is the blank bag correction factor following washing with ND.

The digestibility of DM (DMD) and NDF (NDFD) were calculated using marker concentrations according to the equations:

[3] $DMD, \% = 1 - [M_I/M_E]$

[4] $NDFD, \% = 1 - [M_I/M_E \times NDF_E/NDF_I]$

where M_I is the marker concentration of diet consumed (%), M_E is the marker concentration of the fecal sample (%), NDF_I is the NDF concentration of diet consumed (%), and NDF_E is the NDF concentration of the fecal sample (%).

2.3.3. Statistical Analyses

All statistical procedures were performed using SAS software (SAS Institute Inc., Cary, NC). Residuals of indigestible fractions and calculated digestibilities were checked for normality and outliers. Outliers were removed if their distance from the upper or lower quartile exceeded three times the interquartile range (Tukey, 1977). Indigestible fractions were evaluated by diet and sample type (feed and feces) in accordance with a 2 × 2 × 2 factorial arrangement using PROC GLIMMIX with incubation-animal serving as a random factor in the statistical model. Differences were considered significant at $P \leq 0.05$. Digestibility estimations were assessed by diet utilizing the previous model with animal and sampling period as random factors. Statistical model selections were based upon corrected Akaike's information criterion with variance partitioning of random factors for all models being performed with the Wald Z test. Mean comparisons were performed using the least significant difference for all significant effects ($P \leq 0.05$).

The effect of incubation animal (block) and percent treatment difference upon the required sample size per treatment to obtain adequate statistical power for iDM and iNDF were simulated using a script developed for the R version 3.5.2 software (R Core Team, 2019). Data from both dietary treatments were pooled and analyzed for each method, iDM and iNDF, and sample type, feed and feces, combination. Percent treatment differences were determined from the pooled data set, and blocks (i.e., incubation animals) were assumed to span 2 to 16 in a research setting with the associated variance being determined for each combination from the pooled data. Fixed parameters for the simulation were: $\alpha = 0.05$ (statistical significance), $\beta = 0.90$ (statistical power), treatments = 8, blocks (i.e., incubation animals) ranging from 2 to 16, and treatment difference varying from 5 to 15% and 15 to 25% for feces and feeds, respectively.

2.4. Results

There was no interaction of BT × SA × IL ($P > 0.05$) for iDM and iNDF values or their resultant DMD and NDFD (Table A-2). Data are presented by each significant two-way interaction with main effects being discussed if not present within a significant interaction.

2.4.1. Indigestible Dry Matter

For the iDM residue, feeds demonstrated multiple significant interactions with CT feces having a single interaction, only the CON feces did not exhibit any two-way

interactions. The CON feed demonstrated an interaction of BT × SS with the F58 × 40 mg treatment having the greatest residue and the 40 mg rates demonstrating greater indigestible residue regardless of BT (Table A-3). There were BT × IL interactions for both feed types and the CT feces (Table A-4). For all samples the greatest residue remained within F58 × 288 h with no difference in remaining BT × IL combinations except F57 × 288 h treatments acting as intermediates for both feedstuffs. There was an interaction of SS × IL for the CT feed as the 40 mg × 288 h had the greatest residue whereas 20 mg × 576 h had the least (Table A-5). When exploring the main effects for CON feces, an effect of BT was observed with F58 having greater iDM (Table A-6). In contrast, SS impacted both feces with the 40 mg SS corresponding to 5.48 and 8.7% greater residue for the CON and CT feces.

The resultant DMD and NDFD only demonstrated an interaction for BT × SS within the CON diet, with greater digestibilities being demonstrated within the F58 × 20 mg treatment whereas all other combinations remained similar (Table A-3). Within the CT diet there was an effect of SS upon NDFD as the 20 mg/cm^2 resulted in increased digestibility estimates. However, incubation length appears to have the greatest effect upon digestibility approximations for both diets as the calculated DMD and NDFD were on average 11.5 and 18.9% greater, respectively, within the 576-h incubations.

2.4.2. Indigestible Neutral Detergent Fiber

Similar to the trend illustrated by iDM, for iNDF residue both feeds demonstrated two significant interactions whereas no significant interactions were

present for either feces. There was an interaction of BT × SS for the CON feed with greater iNDF values in the 40 mg treatment irrespective of the bag (Table A-3). Both feeds demonstrated an interaction for BT × IL with the F58 × 576 h having the lowest iNDF residue. However, the CON feed had greater residue when incubated for 288 h regardless of BT, whereas the CT feed residue was greatest in the F57 × 288-h treatment (Table A-4). Only the CT feed had an interaction for SS × IL, trending similarly to iDM with 40 mg × 288 h having the greatest residue and 20 mg × 576 h the least (Table A-5). The main effects of BT affected CON feces with F58 having elevated iNDF (Table A-6). However, SS and IL affected both feces with 40 mg SS increasing residue 7.3% and the 576 h IL reducing iNDF values 9.6%, on average. When comparing coefficients of variation (CV) within sample types, it is evident that iNDF has less variation relative to iDM (Fig. B-1). In general, feed-CT exhibits greater variability regardless of marker type as compared to the CON feedstuff, whereas manure displays much less volatility and a more predictable change in variation from iDM to iNDF.

Subsequent digestibility estimates demonstrated an interaction of BT × SS for DMD and NDFD within the CON diet (Table 3). Use of F58 × 20 mg resulted in an average digestibility increase of 4.7 and 8.2% for DMD and NDFD, respectively. In contrast to iDM, approximations for the CT diet were affected by BT with the use of the F58 bags resulting in 4 to 6% higher digestibility estimates. Length of incubation, however, has similar tendencies to digestibilities calculated from iDM with 576-h incubation corresponding to an average increase in DMD and NDFD of 8.6 and 14.9%.

2.4.3. Variances and Sample Size

Variance partitioning of iDM residue indicated that incubation animal had only a small effect upon residue variability, although the effect was greater upon manure than feed samples (Table A-7). When exploring the partitioning for digestibilities, trial animal accounted for a substantial portion of the variability of DMD, whereas period accounted, on average, for 46.5% of the variation associated with NDFD. The variance partitioning for iNDF residues followed the same trend illustrated by iDM (Table A-7). However, the variability of incubation animal increased within the feed component and decreased in the feces. Partitioning within digestibilities proved volatile as trial animal accounted for a minor portion of the variation in comparison to period for the DMD-CON and NDFD-CT. By contrast, trial animal represented the major source of variability relative to the period for both DMD-CT and NDFD-CON. When reviewing the sample size simulations (Fig. B-2), it is apparent that in our trial feed required far fewer treatment replications to obtain 90% power than feces.

2.5. Discussion

The utilization of iDM, rather than indigestible residues, as an internal marker would be advantageous due to a reduction in analytical procedures (Detmann et al., 2001). However, microbial and non-microbial contamination from ruminal incubation can introduce large, heterogeneous variability (Huhtanen et al., 1994; Valente et al., 2011), reducing the precision of estimates except for situations in which iNDF is a large component of the iDM. Within the current study a reduction in contaminants following

washing in ND solution was evident when comparing iDM and iNDF values of the respective sample and diet combinations. Correspondingly, the vast majority of iNDF residues demonstrated lower CV than those of iDM with an average reduction of 3.5 and 4.8% for feed and feces, respectively. Generally, there is greater variation for iDM relative to iNDF (Valente et al., 2011); although the accuracy of recovery estimates are not commonly affected, the precision is typically reduced when using iDM (Sampaio et al., 2011). Within our study, there was large variation resulting from feed-CT dependent upon treatment, whereas feces-CT was much more stable. The CT present within feed commonly has greater precipitation capacity compared to those in feces (unpublished data), thereby enabling greater inhibition of microbes and associated enzymes, as well as the formation of CT complexes with endogenous/exogenous substrate and microbes (Haslam, 1989). Reduction in precision due to the presence of secondary compounds could potentially influence markers for estimation of intakes, excretion, and digestibilities. Tedeschi and Fox (2018) noted that discrepancies in iNDF greatly influenced TDN values, with tropical feedstuffs commonly penalized to a greater extent than temperate. This could be a consequence of antinutritional factors, such as CT, being more widely distributed in tropical species (Muir et al., 2009).

In general, DMD estimations from iNDF exceeded those of iDM by 0.8 and 1.9%, on average, for the CON and CT diets, respectively. This is consistent with Huhtanen et al. (1994) who showed that iDM provided lower estimates of DMD when compared to iNDF. The NDFD of the CON diet had an average +8.05% change when using iNDF, whereas NDFD of the CT diet only exhibited a +0.07% change on average

when calculated from iNDF. Comparing iDM and iNDF, digestibilities of the CT diet were variable with differences in DMD and NDFD ranging from -3.41 to 3.38% and -4.75 to 3.74%, respectively. It is likely that greater variability and reduced differences in digestibilities are not solely due to indigestible constituents, but rather an artifact of insoluble CT-substrate complexes. A major issue with CT presence is that the ND washing process provides an environment, high heat and pH 7, that induces additional polymerization and does not break CT-substrate complexes, rather there is an increase of insoluble CT complexes (Van Soest, 1994). Due to the variability present within residues and digestibilities of CT samples, when analyzing a sample containing CT, use of multiple sequential washes may be a method of accounting for CT-complex retention for improved precision (Van Soest and Robertson, 1985; Van Soest, 1994).

Collectively, the F58 bags resulted in greater iDM residue values for all samples, largely a result of contamination due to characteristics of the bag material. This is evidenced by F58 bags within BT × SS and BT × IL exhibiting a greater percent change following washing with ND solution. Lower porosity bags (< 10 μm) for in situ methods can result in estimation errors due to reduced microbial influx and micro-environments within the bag (Nocek, 1988; Huhtanen et al., 1998); however, fermentation was not hindered due to bag porosity in our study as feedstuffs within F58 bags had equal or lesser iNDF residues. Since bag porosity did not impact degradation, it appears probable that there was sample washout for feces incubated within F57 bags as evidenced by lower fecal recoveries relative to the F58. This finding is consistent with the greater fecal loss with increased bag porosity noted by Huhtanen et al. (1994).

Similarly, SS had a substantial effect upon residues regardless of marker or diet, with 40 mg resulting in greater indigestible constituents relative to contemporaries. Both BT × SS and SS × IL interactions demonstrated a larger percent change from iDM to iNDF when utilizing the larger SS, but to a lesser degree for manure within BT × SS. Larger SS reduced pore size-to-free surface area ratio that likely resulted in reduced microbial and enzymatic activity, decreasing the rate of degradation (Huhtanen et al., 1998; Vanzant et al., 1998). This would more greatly affect bags containing larger amounts of fermentable substrate that are exposed to the rumen environment for a shorter period of time or are predisposed to higher contamination levels. Although the use of 10 mg/cm^2 is commonly utilized for in situ studies (Vanzant et al., 1998) and may provide more accurate estimates of truly indigestible components, small residues following extended incubation length could potentially introduce more error due to greater analytical precision required (Sampaio et al., 2011).

Incubation length followed a similar trend irrespective of BT, with increased ruminal residence providing decreased residue estimates even in the presence of contaminants. However, greater discrepancies were present for SS × IL interaction as larger SS resulted in elevated residues for both IL. In general, the 576-h incubations exhibited lower, more homogenous estimates of residue that yielded substantially higher digestibility approximations. This is in agreement with Koukolová et al. (2004) who reported considerably greater degradation when using 504-h incubations vis-á-vis 168 h. The 576-h incubations appear to better represent the indigestible constituents as digestibility estimates using iDM and iNDF varied only slightly in most instances,

whereas 288-h estimates demonstrated variability indicative of digestible material removal during ND wash. However, an increase in contamination with prolonged incubation length was evident as the percent change from iDM to iNDF was generally lower for 288- versus 576-h incubations. Therefore, caution must be taken when utilizing iDM. In our study 288-h incubations do not appear adequate for the determination of truly indigestible constituents or precise digestibility estimations when utilizing 20 and 40 mg/cm^2 SS-to-surface area ratio.

The required sample size for adequate statistical power was greatly affected by sample type, as a reduction in treatment differences within the feces restricted the power obtained relative to the that of the feed. The divergence between feed and feces is a consequence of the total fermentable substrate present within each sample type. The use of a concentrate diet within our study provided a large amount of potentially fermentable substrate that enabled larger treatment differences to be observed; however, greater sample size (replication) should be utilized for the determination of indigestible contents of roughage diets due to the lower total fermentable substrate that could result in less prominent treatment differences. Although the degree of treatment difference had the greatest effect upon attaining adequate sample size, the number of blocks introduced variation that could have a substantial impact on results. We used an average sample size of 90 bags per treatment with an average treatment difference of 22.5% for iDM and iNDF feed residues, exceeding the requirements to detect a true difference if present. However, for feces, our design did not enable 90% statistical power to be attained due to treatment differences ranging from roughly 8 – 10%. Based upon our data, if a 25%

difference between feed treatments is sought and only two incubation animals are available, then a total of 24 bags per treatment (12 bags per incubation animal) is required. This level of replication greatly exceeds the triplicate and quadruplicate replications per incubation animal that is often used for in situ experimentation. When comparing iDM and iNDF of feces, iNDF reduced the sample size required by decreasing incubation animal variation and increasing treatment differences. For feed residue a greater sample size is needed for iNDF than that of iDM, due to greater incubation animal variance and lesser treatment differences.

In summary, our results indicate less error and greater precision in the determination of indigestible components using iNDF. Estimates of iDM are largely influenced by contamination that can vary greatly based on intrinsic factors associated with the bag and incubation duration. However, the presence of CT appears to influence both iDM and iNDF to varying degrees. In agreement with Krizsan et al. (2015), in-situ determination of indigestible residues should be performed using bags with a pore size ranging from 6 to 12 μm as the use of F57 bags (25 μm) resulted in lower fecal recovery as compared to F58 bags (10 μm). Within our study, SS-to-surface area ratio of 20 mg/cm^2 is best suited for determination of indigestible components as 40 mg/cm^2 appears inhibitory to degradation, promoting larger residues that were not corrected when using 576-h incubations. However, the determination of indigestible residues utilizing 10 mg/cm^2 could potentially reduce the incubation length required and the accompanying increase in contamination, but error associated with analytics must be accounted for. Lower indigestible residues resulted from 576-h incubations relative to

those of 288 h. This indicates that pdNDF is still available after 288 h within the rumen, albeit a function of IL and SS. We found that improved estimation of indigestible components can be attained through the determination of iNDF using F58 bags, 20 mg/cm^2 SS, and 576 h IL. When utilizing indigestible components, the determination of associated power for a specific sample type is a prerequisite. The assumption that two incubation animals and low treatment replication will suffice appears inappropriate for indigestible residues, as sample numbers need to be increased 3-to-4 fold when utilizing similar sample types to obtain adequate power for determination of differences. Further research should investigate the impact of secondary compounds beyond CT upon accuracy and precision of estimates attained using internal markers. A better understanding of SS × IL combination dynamics is needed prior to the development of a standardized methodology.

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3. INFLUENCE OF QUEBRACHO TANNIN EXTRACT FED AT DIFFERING RATES WITHIN A HIGH-ROUGHAGE DIET ON THE APPARENT DIGESTIBILITY OF DRY MATTER AND FIBER, NITROGEN BALANCE, AND FECAL GAS FLUX

3.1. Overview

Ruminant production is essential in meeting the requirements of high-quality protein of an increasing global population. However, gaseous byproducts from ruminant production such as methane (CH_4) and nitrous oxide (N_2O) can reduce energy efficiency and be detrimental to the environment. The major sources of agricultural non-CO_2 emissions are enteric fermentation and manure, accounting for 40 and 15% of total emissions, respectively. Public awareness and concern regarding antimicrobial resistance and chemical residues of feed additives have prompted the pursuit of natural rumen modulators. Condensed tannins (CT) are an alternative method of improving animal and system-level efficiency due to their potential for improving protein use efficiency and reducing CH_4. In this study, we evaluated how quebracho tannin (QT) extract inclusion at rates, 0, 1.5, 3, and 4.5% of dietary dry matter (DM), within a roughage-based diet affected apparent digestibility, N balance, ruminal parameters, and fecal gas emissions. The 4.5% inclusion rate reduced DM intake and increased fecal DM production ($P \leq 0.01$). Addition of QT affected all digestion coefficients ($P < 0.01$) with a linear reduction as QT level increased. Reduced digestibility led to greater daily energy excretion with increasing QT inclusion ($P < 0.01$), resulting in a linear reduction in daily

digestible energy (DE), DE per kg DM intake, and DE:gross energy. There were greater daily fecal energy and N output with increased QT ($P < 0.01$), but no difference in concentrations. Feeding QT resulted in a linear shift in N excretion from urine to feces. For fecal gas flux, cumulative CO_2 and N_2O decreased linearly with increased QT supplementation; by contrast, CH_4 displayed a cubic relationship with the 3% treatment having the lowest emissions. Total CO_2 equivalents (CO_2e; $CH_4 + N_2O$) displayed a cubic relationship with emissions being largely driven by CH_4 production ($r = 0.99$, $P < 0.01$). In contrast, gross CO_2e (Total $CO_2e + CO_2$) displayed a linear reduction in emissions with increased QT, with CO_2 having the largest influence ($r = 0.99$, $P < 0.01$). Emission factors displayed a linear decrease ($P < 0.05$) in manure N emitted as N2O-N with increased QT inclusion. We concluded that feeding QT above 1.5% is likely to reduced fecal CO_2 and N_2O emissions but also apparent digestibility and N balance. Further determination of energy fractionation and urinary gas emissions is required to fully define CT influences on system efficiency.

3.2. Introduction

Ruminant production utilizes land areas unfit for cultivation since the conversion efficiency of energy and protein from vegetative plants greatly exceeds the nutritional value of what is consumed, with corresponding energy and protein values commonly increasing by factors of 3 and 6, respectively (CAST, 1999). This makes ruminants an important source of human edible protein and essential nutrients to meet the 2050 global food demand estimates (FAO, 2017; White and Hall, 2017). However, gaseous

byproducts from ruminant production such as methane (CH_4), nitrous oxide (N_2O), and ammonia (NH_3), reduce the animal's energy efficiency, production efficiency, and are ultimately detrimental to the environment (Tedeschi and Fox, 2018). In terms of global agricultural non-CO_2 emissions, enteric fermentation and manure on pasture accounted for approximately 40 and 15% of total emissions, respectively, in 2010 (Smith et al., 2014; Tubiello et al., 2014).

Numerous feed additives have successfully improved growth efficiency and emission status of ruminants, but contemporary concerns regarding antimicrobial resistance and chemical residues have prompted the pursuit of natural rumen modulators (Patra and Saxena, 2010). Condensed tannins (CT) are a diverse group of naturally occurring secondary metabolites that are potential alternative feed additives due to their reactivity when in proximity to proteins, carbohydrates, microbes, and enzymes (Haslam, 1989). Within ruminant nutrition, CT are recognized for reducing feed intake and fiber digestibility (Waghorn and McNabb, 2003) but can potentially improve nutrient efficiency through protein sparing and CH_4 mitigation (Waghorn et al., 1987), making it plausible that feeding CT to ruminants could reduce environmental impacts and improve system-level efficiency. The objective of this study was to determine the effect of differing rates of quebracho tannin inclusion within a roughage-based diet upon apparent digestibility, nutrient metabolism, ruminal parameters, and resultant manure gas emissions in beef steers.

3.3. Materials and Methods

The animals used in this experiment were registered and cared for according to guidelines approved by the Institutional Animal Care and Use Committee (AUP 2017-0306) at Texas A&M University.

3.3.1. Metabolism Experimental Design

A 4 × 8 Latin rectangle design utilizing four periods and 8 English crossbred steers (435 ± 17 kg BW) were used to determine the effects of quebracho (*Schinopsis balansae*) CT (QT; SILVATEAM, San Michele Mondovi Italy) at 0, 1.5, 3, and 4.5% of DM (QT_0, $QT_{1.5}$, QT_3, and $QT_{4.5}$), so that each treatment was replicated by two animals within each period. The Large Ruminant Nutrition System (http://www.nutritionmodels.com/lrns.html; accessed 24 April 2018; Tedeschi and Fox, 2018) was used to formulate a roughage-based total mixed ration (Table A-8) to meet maintenance requirements, with the addition of QT serving as dietary treatments. Animals were provided the base diet at 1.65% body weight (BW), DM basis, with pre-weighed QT being hand mixed into individual animal feed prior to provision once daily at 0800 h. Animals were housed in a climate-controlled room within individual stalls (1.8 x 3 m) fitted with a feed bunk and allowed free access to water. Temperature and relative humidity were monitored using digital HOBO loggers (Onset Computer Corporation, Model# UX100- 003) and water intake was measured using analog water meters (Neptune Technology Group, Inc., Model# T10-DR-075-G-F). For each period, dietary adaptation spanned 12 d followed by relocation to metabolism crates for total

fecal and urine collection over 4 d. Prior to the trial initiation and upon removal from metabolism crates for each subsequent period, animals were fasted for 18 h and then weighed to determine shrunk BW (SBW) followed by adaptation to succeeding diets.

3.3.2. Sample Collection, Preservation, and Analyses

Batch samples (250 g) of the base diet were collected daily for the last 10 d of each period. Individual animal orts and feces were weighed, homogenized, and subsampled daily before feeding and were stored in a -20° C freezer. Daily urine samples were collected using polypropylene vats containing 300 mL of 3 N HCl solution to acidify urine and prevent NH_3 volatilization. Daily urine was weighed and two 50-mL subsamples were stored at -20°C.

Total urinary N analysis was performed by Servi-Tech laboratories (Amarillo, TX) using the Dumas combustion method (Method# 990.03; AOAC, 2000). All whole samples were dried at 55° C for 72 h (or until weight loss ceased) then ground to pass through a 2-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ) and composited by animal and period based upon the proportion of daily orts and fecal output. A 50-g subsample of each composite was shipped to Cumberland Valley Analytical Services (CVAS; Waynesboro, PA) for chemical analysis of dry matter (DM; Goering and Van Soest, 1970), neutral detergent fiber with addition of amylase and sodium sulfite (aNDF; Van Soest et al., 1991), acid detergent fiber (ADF; Method# 973.18) (AOAC, 2000), lignin, crude protein (CP; Method# 990.03; AOAC, 2000) in a Leco FP-528 Nitrogen Combustion Analyzer (Leco Corporation, St. Joseph, MO),

soluble CP (Krishnamoorthy et al., 1982), non-fibrous carbohydrates (NFC), fat (Method 2003.05;AOAC, 2006), starch (Hall, 2009), sugar (Dubois et al., 1956), a complete mineral panel (Method# 985.01; AOAC, 2000) in a Perkin Elmer 5300 DV ICP (Perkin Elmer, Shelton, CT), and calculation of total digestible nutrients (TDN) and net energy (NE). Gross energy (GE) was measured on feed, fecal, ort, and urine samples using a bomb calorimeter (Parr adiabatic calorimeter; Parr Instruments Co., Moline, IL) for determination of fecal energy (FE), urinary energy (UE), and digestible energy (DE).

On the last day within metabolism crates, blood samples were collected via jugular venipuncture before the morning feeding using 10-mL red top serum vacutainers (Becton, Dickinson and Co. Franklin Lakes, NJ). Samples were immediately chilled, and serum was obtained by centrifuging at 3000× *g* for 20 min at 9°C, the supernatant was aliquoted into polypropylene tubes and stored at -20°C. Samples were analyzed for glucose, albumin, blood urea nitrogen (BUN), creatinine, and total protein by Texas A&M Veterinary Medical Diagnostic Laboratory using commercial test packages and an AU480 analyzer (Beckman Coulter; Brea, California). Upon removal from metabolism crates, steers had 500 mL of rumen fluid collected via an esophageal tube connected to a vacuum pump. Rumen inoculum was filtered through 8 layers of cheesecloth and pH was taken immediately. Inoculum samples were allocated into duplicate containers for the preservation of volatile fatty acids (VFA), NH3-N, and protozoa. Preservation methods were 8 mL of inoculum and 2 mL of 25% (wt/vol) metaphosphoric acid solution for VFA analyses, 2 mL of inoculum to 8 mL of 0.1 N HCl acid solution for NH3 analyses, and 1 mL inoculum and 10 mL of ethanol for protozoa counts, all

samples were stored at -20°C. Concentrations of NH3-N were determined by colorimetric methods and VFA using gas chromatography (cite). Protozoa counts were determined by methods described by Dehority (1984) without staining. Using a Sedgewick Rafter counting chamber, protozoa within a 1-ml aliquot of sample were counted using Nikon Eclipse E200 microscope (Nikon Corporation Tokyo, Japan) at 100x magnification with a 0.5-mm square counting grid; 25 evenly spaced grids from the entire chamber surface were counted, and an average was computed for each rumen fluid sample.

3.3.3. Fecal Gas Flux Feeding, Fecal Sampling, and Analyses

Upon cessation of the metabolism trial, the eight animals were utilized to determine the effect of QT upon cattle fecal gas flux, using the same diet and treatments from the metabolism trial. Two steers were randomly assigned to each dietary treatment (QT_0, $QT_{1.5}$, QT_3, and $QT_{4.5}$). Animals were housed outside, by treatment, within pens (9.1 × 12.2 m) fitted with Calan-gate feeders (American Calan, Northwood, NH) and waterer, with feeding occurring once daily at 0800 h and free access to water. Animals were adapted to diets for 12 d before collection of fresh feces over two days. This trial spanned June and July 2017 in College Station, TX, USA with the study area consisting of Boonville, Rader, and Zulch fine sandy loam soil series with a mean annual precipitation of 914 to 1118 mm and a frost-free period of 260 to 290 d (NRCS, 2002). Meteorological data were collected from the local municipal airport with instantaneous soil moisture and volumetric water content being measured prior to all gas collections

using a 5TM soil probe and the ProCheck hand-held read out device (Decagon Devices, Inc., Pullman, WA). At the time of sampling the mean ambient temperature was 28.4°C with 76% relative humidity and an atmospheric pressure of 1004 hPa. The mean soil moisture and temperature during gas sampling was 0.045 m^3/m^3 and 31.9°C, respectively, with soil moisture near the permanent wilting point for the soil type (Ratliff et al., 1983).

Fresh feces were collected by visually observing animals and immediately collecting manure following a witnessed defecation event. Individual animal feces were placed within storage bags and stored at 4°C. Following the 2-d collection period, individual animal feces were homogenized and pre-weighed for placement within the respiration chamber collars the following day. Excess manure was prepared for chemical analyses by drying at 55°C for 72 h. Samples of the base diet were collected daily for 4 d prior to fecal collection and dried at 55°C for 48 h then ground to pass through a 2-mm screen. A 50-g subsample of feed and fecal samples were shipped to CVAS for chemical analysis as outlined previously.

3.3.4. Gas Collection

Gases were collected using vented static chambers according to the protocols of Parkin and Venterea (2010) and de Klein and Harvey (2012). The chambers were constructed from polyvinyl chloride pipe with the collar having an area of 324 cm^2 (Parkin and Venterea, 2010) providing a chamber area-to-perimeter ratio of 19.7 cm (Rochette and Eriksen-Hamel, 2008). Chambers consisted of two parts, soil collar and

chamber cap. Two weeks prior to initial gas collection soil collars were permanently placed for the duration of the trial with 10 cm inserted into the soil and 10 cm protruding above the soil surface; whereas chamber caps were only placed on collars for the duration of gas sampling. The chamber cap was fitted with a vent tube and sampling port that housed a butyl septum, as well as a rubber gasket and outer seal to mitigate gas leakage.

A total of 18 collars were placed in 2 rows of 9 (8 feces and 1 background soil flux) with collars within a row 50 cm apart and 2-m row spacing. On Day 0, within each row, 900 g of wet feces from each animal was placed on the soil surface within individual collars according to a predetermined random assignment. There were two rows to replicate feces from each animal, and each treatment was replicated twice within each row. Daily gas collections were made by row with each sampling day utilizing the same progression within and between rows.

Gas collection occurred on Days 0, 1, 2, 4, 6, 8, 10, 12, 14, 17, 21, 24, 28, 31, and 35 following feces application. Collections occurred between 0900 and 1100 h to represent daily mean flux using deployment times of 0, 12, 24, and 36 min following chamber cap placement. For each deployment time, gas samples were collected using airtight glass syringes fitted with a two-way stopcock, whereby a 20-mL sample was injected into pre-evacuated 10-mL exetainer vial (Labco Limited, Lampeter, Ceredigion, UK). Following collection, gas samples were stored at 4°C prior to analyses using a gas chromatograph equipped with electron capture, flame ionization, and thermal conductivity detectors for determination of CO_2, CH_4, and N_2O (Holland et al., 1999).

Daily mass-based fluxes of CO_2, CH_4, and N_2O were determined from the slope of the mass concentration (μg/cm^3) vs time (h) curve (Parkin and Venterea, 2010). Multiplying the slope (μg/h) by the chamber volume (cm3) and then dividing by the surface area (cm^2) results in flux units of μg/cm^2/h. Linear regression was used to calculate slope when the slope was constant over time, while quadratic regression was utilized when the slope fluctuated over time. Gas concentrations below the minimum detectable limit of the gas chromatograph for individual gases were assumed to have zero flux and gas fluxes were extrapolated to represent daily emissions. Cumulative gas production was calculated as the sum of all collections without interpolation. The calculation of CO_2 equivalent emissions (CO_2e) was performed using 100-year global warming potentials for CH_4 and N_2O, 28 and 265, respectively, by multiplying the raw gas value by the corresponding global warming potential (IPCC, 2014). Emission factors for N_2O were calculated as the cumulative N_2O-N emitted as a proportion of the N within manure (Sordi et al., 2014). Gas production, total CO_2e, and gross CO_2e were evaluated per unit of fecal DM incubated, whereby total CO_2e is the sum of CO_2e for CH_4 and N_2O and gross CO_2e is the sum of CO_2 and total CO_2e. Mean gas emissions and individual animal data from the metabolism trial were integrated to calculate the total primary gas fluxes for daily fecal excretion.

3.3.5. Statistical Analyses

All statistical procedures were performed using SAS software (SAS Institute Inc., Cary, NC). Metabolism variables were evaluated by a Latin design using PROC

GLIMMIX, and animal and period were considered random variables. Mean comparisons were performed using the LSMEANS statement with the adjustment proposed by Tukey-Kramer for all significant effects. Significance was declared at $P \leq 0.05$ and tendencies were assumed at ($P \leq 0.10$). Polynomial contrasts were performed for all variables to determine linear, quadratic, and cubic effects of CT inclusion in the diet.

Gas flux data were analyzed using PROC MIXED with animal nested within treatment as the random factor. Residuals of the gas flux data were checked for homoscedasticity and normality, whereby cumulative gas production exhibited unequal variances. To account for heteroscedasticity, the denominator degrees of freedom were adjusted with the SATTERTHWAITE option, and in the REPEATED statement the GROUP option was used to estimate treatment variances separately. The largest standard error of the mean is reported. Mean comparisons were performed using the least significant difference for all significant effects ($P \leq 0.10$) using the LSMEANS statement with the PDIFF option. Polynomial contrasts were performed for all variables to determine linear, quadratic, and cubic effects of CT inclusion in the diet. Correlation coefficients for gases and manure nutrients, N, soluble N, NDF, ADF, and NFC, were determined using PROC CORR. Daily animal emission estimates from metabolism data were analyzed using PROC GLIMMIX with animal and period as random factors following a Latin square design, and mean comparisons were performed using LSMEANS and the Tukey-Kramer adjustment for all significant effects ($P \leq 0.05$).

3.4. Results and Discussion

3.4.1. Metabolism

Table A-9 shows the effect of QT upon intake and excretion profiles. There was no dietary effect upon daily water intake or the water-to-dry matter intake (DMI) ratio, although $QT_{4.5}$ did exhibit elevated water consumption relative to all other treatments. The effect of CT upon water intake is consistent with previous research. Landau et al. (2000) noted a difference in total water consumption yet feed-to-water intake ratio did not differ. Supplementation of $QT_{4.5}$ had the lowest DMI ($P = 0.018$) and greatest fecal production ($P < 0.001$). Dry matter intake responded in a quadratic manner to QT inclusion; while increased QT inclusion resulted in a linear increase in fecal production. Reduced DMI is common when CT is supplemented due to associated astringency and decreased ruminal digestibility (Landau et al., 2000; Frutos et al., 2004), with a resultant increase in fecal production commonly seen in roughage-based diets (Ahnert et al., 2015; Aguerre et al., 2016). Within our trial, intake was only reduced at the highest QT level. However, this was largely dependent upon the individual animal as most had no orts following initial exposure to new diets. Urine production tended ($P = 0.07$) to differ across QT inclusion rates with a reduced value for QT_3 compared to all other treatments.

The addition of QT affected all digestion coefficients ($P < 0.001$), with DM, NDF, ADF, and N digestibilities exhibiting a linear reduction with increased QT level (Table A-10). Apparent N digestibility demonstrated a greater reduction than DM or NDF, which is consistent with previous research utilizing QT (Aguerre et al., 2016). High levels of unbound CT can depress fibrous carbohydrate degradation via direct or

indirect inhibition of microorganisms and associated enzymes (Barry and Manley, 1984; Patra and Saxena, 2009). Supplementation of QT above 1.5% of the DM decreased DMD and OMD in cattle fed lower-quality grass (Piñeiro-Vázquez et al., 2017). However, the effect upon digestibility is variable and likely dependent upon the feedstuff as QT at 3% DM did not reduce DMD, NDFD, or ADFD in forage diets consisting of alfalfa hay and corn silage (Dschaak et al., 2011). It is likely that diets of lower protein and readily fermentable carbohydrate allow greater unbound CT to enter the rumen and directly inhibit microbial processes (Barry and Manley, 1984), such as the case in the current study. The reduction in digestibility led to greater total daily energy excretion with increased QT rate ($P \leq 0.016$), resulting in a linear reduction in daily DE, DE/kg DMI, and DE:GE as QT rate increased (Table A-11). Decreased DE-to-GE ratio with increased CT provision has been observed previously for beef and dairy cattle (Grainger et al., 2009; Piñeiro-Vázquez et al., 2017). Fractionation of daily GE losses did not demonstrate differences for UE; however, there was more daily FE with increased QT ($P < 0.001$), but no difference in fecal energy concentration. Addition of QT resulted in greater FE as a proportion of total energy excreted ($P = 0.004$) with FE-to-UE ratio increasing with inclusion rate ($P = 0.015$).

When comparing urinary N profiles, there was no difference in the daily amount excreted or concentration of N; however, there was a linear reduction in daily urinary N excretion with increased QT ($P < 0.001$; Table A-10). In contrast, there was a linear increase in daily fecal N with greater QT inclusion ($P < 0.001$). There was no difference in fecal N concentration, but a strong quadratic relationship was present with $QT_{1.5}$

having the greatest N concentration relative to all others. The supplementation of QT resulted in a shift in N excretion from urine to feces in a linear fashion ($P = 0.007$) with the proportion of total N excreted within the feces increasing 19% on average for the two highest QT rates. This resulted from the reduced apparent N digestibility as a result of QT in the diet. Changing the route of N excretion is common when feeding CT due to reduced ruminal proteolysis (Waghorn et al., 1987; Orlandi et al., 2015). Shifting excretion of N to the feces can potentially be beneficial from an environmental and production standpoint as fecal N is less volatile than urinary N due to reduced degradation rate that results in air pollutants, NH_3 and N_2O, possibly reducing emissions and improving N cycling (Ndegwa et al., 2008; Patra and Saxena, 2011). Although QT affected apparent N digestibility, there was no difference in N retention or total N excretion, but a linear reduction in N retention with increased QT was present ($P = 0.057$). Nitrogen retention was relatively similar with the exception of $QT_{4.5}$, which resulted in a 39% decrease compared to the average of other treatments. The lack of significance is due to period accounting for 46% of the variance; however, there was a noticeable among-animal effect irrespective of treatment, although this was not detected within the variance partitioning. The protein-sparing effect that is often associated with CT is short-lived and possibly increases the protein requirements of the animal due to endogenous protein loss in response to CT consumption (Tedeschi and Fox, 2018). During the collection of manure, mucal casts became evident in the feces from animals on the $QT_{4.5}$ treatment. Mucal cast excretion has been reported in sheep fed QT (Hervás et al., 2003). Condensed tannins may be locally toxic to surface epithelium of the

gastrointestinal tract as epithelial degeneration, ulceration, and increased mucosal histiocytes have been noted in animals fed CT (Dawson et al., 1999; Hervás et al., 2003).

Level of QT inclusion did not have an effect on ruminal parameters (Table A-12). Inclusion rate did not affect ruminal pH, but a quadratic tendency ($P = 0.062$) was present with QT_0 and $QT_{4.5}$ having the lower pH relative to other treatments. There was a tendency for decreased protozoa numbers for $QT_{1.5}$, with a cubic effect being exhibited. The capacity for QT to reduce protozoa numbers appears low, agreeing with Benchaar et al. (2008) who reported no effect upon total protozoa or individual genera. In contrast, Vasta et al. (2010) reported an increase in total protozoa in sheep. The effect of CT upon protozoa counts is variable with reports of decreased protozoa with in vitro methods which do not fully encompass the dynamic environment of the rumen (Makkar and Becker, 1995; Tan et al., 2011), yet feeding of pistachio extract at 5 to 15% did result in reduced protozoal numbers in vivo (Jolazadeh et al., 2015). A tendency for QT to reduce ruminal NH3-N was present ($P = 0.088$) with a linear reduction ($P = 0.014$) as QT increased. Previous research has demonstrated the capacity for CT to decrease ruminal NH_3-N production by reducing ruminal proteolysis (Cieslak et al., 2012; Jolazadeh et al., 2015); however, the effectiveness of QT is inconsistent as no difference (Benchaar et al., 2008; Mezzomo et al., 2011) and reduced (Ishlak et al., 2015; Aguerre et al., 2016) NH_3-N levels have been observed. Dietary treatment did not affect total VFA, individual VFA, or acetate-to-propionate ratio; however, there was a cubic trend ($P = 0.018$) for isovalerate, as well as linear tendencies for isobutyrate ($P = 0.098$) and butyrate ($P = 0.107$) (Table 5). These results are similar to previous reports noting no

effect of QT upon total VFA production and only minor effects upon branched-chain VFA (Benchaar et al., 2008; Aguerre et al., 2016). Even so, the slight reduction observed in branched-chain VFA could be indicative of reduced amino acid deamination, which agrees with the reduced ruminal NH_3-N as QT increased, or a shift in ruminal bacterial populations that synthesize branched-chain amino acids and convert them to branched-chain VFA (Tedeschi and Fox, 2018). Furthermore, because of the interrelation of isoacids and cellulolytic microbes, decreased branched-chain VFA can be indicative of reduced structural carbohydrate degradation (Andries et al., 1987; Moharrery and Das, 2001), as was seen within our study. No effect was seen for blood parameters, except BUN that had a linear reduction ($P = 0.007$) with increased QT rate ($P = 0.031$). A reduction in BUN has previously been observed in dairy cattle when feeding QT due to decreased ruminal NH_3-N production, which is consistent with our results (Benchaar et al., 2008; Aguerre et al., 2016).

3.4.2. Fecal Gas Flux

There were no treatment differences for fecal DM, N, soluble N, NDF, ADF, or NFC ($P > 0.10$; data not shown). Cumulative production of all gases g DM-1 differed ($P < 0.063$) as a function of dietary QT (Table A-13). Cumulative CO_2 ($P = 0.017$) and N_2O ($P < 0.001$) gas production decreased linearly as QT supplementation increased. In contrast, CH_4 displayed a cubic trend ($P = 0.032$) with QT_3 having the lowest emissions. There was a positive correlation between CO_2 and N_2O ($r = 0.74$; $P < 0.01$; Table A-14), which is commonly seen on a site level when observing soil fluxes associated with

organic carbon and N within the substrate (Xu and Zhou, 2008). Production of CO_2 was related to soluble N and NFC (Table A-14). Jost et al. (2013) observed increased CO_2 production in diets with greater available N and lower ADF, as a result of a greater microbial turnover. Methane was negatively associated with NDF and ADF (r = -0.55 and -0.52; $P < 0.09$), consistent with Külling et al. (2002) who reported an increase in slurry CH_4 with increased degradable fibrous constituents. Previous research has observed lower fecal CH_4 yield from roughage diets relative to concentrates due to increased fermentable substrate providing precursors for methanogenesis (Hashimoto et al., 1981); however, no association with NFC within the feces was observed in the current study. Utilization of feed additives, which result in microbial inhibition within the rumen can potentially increase fecal CH_4 due to greater readily degradable components within the feces. However, feedstuffs with low rumen degradability due to intrinsic properties, such as abundant crosslinked hemicellulose and lignin fractions, will not have increased fecal CH_4 emissions in the short-term due to fermentable substrate remaining unavailable to microbes (Hindrichsen et al., 2005). Within our experiment there were no differences in fecal NDF, ADF, or NFC concentrations across treatments, yet there was a cubic effect for CH_4 production. This erratic trend could result from inconsistent binding to fermentable substrate, or simply an artifact of high variability among animals and our limited sample size.

Total CO_2e exhibited a cubic response ($P = 0.032$) with QT_3 having the lowest emissions (Table A-13). Total CO_2e was largely driven by CH_4 production (r = 0.99, $P < 0.01$). Although the global warming potential of N_2O is considerably greater than that of

CH_4, the cumulative emissions of N_2O were meager in comparison with average CH4 and N_2O fluxes, 253 and 1.5 µg/g DM, respectively. In contrast to total CO_2e, gross CO_2e displayed a linear reduction in emissions with increased QT supplementation, in response to higher CO_2 values ($r = 0.99$, $P < 0.01$). Both total and soluble N emission factors displayed treatment differences ($P < 0.05$) with a linear decrease in manure N emitted as N_2O-N with increased QT inclusion. There was a positive correlation for N_2O and soluble N ($r = 0.67$, $P < 0.01$); however, the soluble N emission factor denoted that less soluble N was lost as N_2O-N with increased dietary QT. A positive correlation between soluble N and NFC ($r = 0.76$, $P < 0.01$) was found. Previous research has observed reduced N_2O-to-N_2 ratios when soluble carbon sources are available due to using N_2O as an electron acceptor by denitrifiers (De Wever et al., 2002; Cardenas et al., 2007). We did not enumerate N_2; however, there tended to be a positive relationship between N_2O and NFC ($r = 0.42$, $P = 0.13$) suggesting that N_2O consumption did not occur or was minimal. As manure-derived N_2O is primarily produced via microbial nitrification and denitrification that are highly dependent upon reductase enzymes (Maeda et al., 2010; Waldrip et al., 2016), it is probable that the reduced emission factors are a result of QT directly or indirectly inhibiting substrate utilization by microbes.

When integrating daily excretion of individual animals within treatments, emission trends decreased in magnitude. There was a linear reduction ($P = 0.02$) in CO_2 emissions with increased QT supplementation; however, the mean separation did not indicate differences (Table A-15). Methane maintained a cubic effect, but a linear trend

also became evident with QT_0 and QT_3 having the least emissions ($P < 0.001$). Similar to CH_4, N_2O maintained a strong linear trend with reduced emissions as QT rate increased ($P < 0.001$); however, a cubic effect was also observed ($P < 0.001$). Total CO_2e paralleled CH_4 with linear and cubic trends ($P < 0.001$) observed with QT_0 and QT_3 having the lowest emissions ($P < 0.001$). Gross CO_2e did not maintain the trend observed for gas flux on a DM basis; rather, a shift occurred as $QT_{1.5}$ demonstrated the greatest overall emissions ($P = 0.015$) with linear and cubic trends ($P = 0.01$). Due to greater daily fecal output with increased QT provision, emission trends diminished or were greatly altered relative to gas fluxes on a DM basis. However, the ability of QT to reduce fecal N as N_2O did not change as less N_2O-N was emitted with increased QT ($P < 0.001$) in a strong linear fashion.

3.5. Conclusions

Feeding QT to steers greatly altered their metabolic parameters, with most demonstrating linear tendencies with increased QT inclusion. The utilization of $QT_{4.5}$ is not recommended under normal conditions as this rate is considerably greater than levels commonly utilized in feeding trials, resulting in reduced digestion coefficients and potential gastrointestinal distress. Relative to QT_0, treatment $QT_{1.5}$ did not differ for metabolic factors but provides the prospect of improved N_2O emission status through excreta profile alteration. In our study, dietary QT decreased N efficiency with possible health implications becoming evident at the highest rate. The reduction in digestibility of fibrous constituents seen at 3 and 4.5% QT rates would be very deleterious to production

when roughage sources comprise the majority of energy within the diet. However, since the determination of CH_4 and heat production were not possible and equations would not represent altered rumen dynamics, speculation upon the impact of QT on energy partitioning was not feasible. Determination of how QT affects energetic efficiency is required. However, it is unlikely that a reduction in enteric CH_4 or heat production could offset the reduced digestibility seen at the two highest supplementation rates.

For fecal gas flux, feeding QT greatly influenced emissions. The flux of CO_2 and N_2O, as well as emission factors, were greatly reduced with QT inclusion; however, CH_4 displayed an erratic trend that could potentially be due to high inter-animal variability. Comparison of gross CO_2e per unit DM demonstrated that QT inclusion reduced emissions. When accounting for total daily excretion, the effect of QT upon fecal gas emissions was diminished; however, these estimates are crude since fecal gas fluxes were not representative of the entire sample population and did not include total excreta emissions as urinary N, which represents the greatest proportion of N lost as N_2O-N, was not accounted for. It is also unknown if supplementation of QT affects urinary N emission by altering N form, making an estimation of urinary N_2O from equations unreliable. The utilization of QT has the potential to reduce fecal gas flux; however, determination of proper supplementation rate is required as the coinciding reduction in emissions and digestibility with increased QT rate results in greater fecal excretion that is not overcome by the level of gas reduction observed in the current study.

In summary, our results demonstrate that CO_2, not CH_4 or N_2O, represents the vast majority of emissions produced from feces within the current setting. Within the

agricultural sector CH_4 and N_2O are the predominant greenhouse gases, but from a total greenhouse gas perspective CO_2 represents 72 to 76% of emissions on a CO_2e basis with agriculture, forestry, and other land uses contributing 14% of CO_2 emissions (IPCC, 2014). Therefore, it is pertinent when comparing methods of improving system efficiency that CO_2 should not be discounted when calculating CO_2e. Disregarding CO_2 could result in a misperception of efficiency associated with practices. Future research investigating fecal gas flux is required and should utilize a larger number of animals as replicates because of the presence of large inter-animal and spatial variability. Greater knowledge of how excreta composition and resultant emissions are altered when utilizing rumen modulators is needed for improving whole-animal emission status and nutrient cycling. The utilization and integration of excretory and respirometry data are required for the advancement of sustainable production practices. In addition, assessing production efficiency as human-edible nutrient on a CO_2e per unit nutrient basis may point the way to practices with greater whole-system efficiency.

3.6. References

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4. EFFECT OF QUEBRACHO (*SCHINOPSIS BALANSAE*) TANNIN EXTRACT FED AT DIFFERING RATES WITHIN A HIGH-ROUGHAGE DIET ON SEASONAL FECAL GAS FLUX

4.1. Overview

Ruminants are required for efficient production of human-edible protein to meet the nutrient demands of an increasing global population. Naturally occurring gaseous byproducts of ruminant production systems, such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), can negatively affect the environment. Apart from enteric fermentation, manure on pasture is the largest non-CO_2 emissions contributor accounting for up to 15% of global agricultural non-CO_2 emissions in 2010. The ability for condensed tannins (CT) to alter fermentation pathways and interact directly with microbial cell membranes suggests that these compounds are plausible alternative rumen modulators for improved nutrient and environmental efficiency through protein sparing, CH_4 mitigation, and shifting N excretion to the feces. Currently the effect of CT supplementation upon fecal gas emissions is relatively unknown. We evaluated how quebracho (*Schinopsis balansae*) tannin (QT) extract fed at 0, 1.5, 3, and 4.5% of dry matter (DM), within a roughage-based diet affected fecal gas emissions at multiple latitudes (College Station and Stephenville, TX) during two periods corresponding to winter and spring. Gas fluxes for CO_2, CH_4, and N_2O were collected over 39 d and cumulative fluxes calculated using interpolation. Random coefficients model with animal nested within dietary treatment and period as the random factor was calculated by

location; Pearson correlations were employed to investigate the association of environmental parameters and fecal nutrient composition with gas fluxes. There was a moderate correlation ($r = 0.33$; $P < 0.01$) between location and soil moisture with a weak association of location and soil temperature. Daily CO_2 flux was influenced by soil moisture and temperature ($r = 0.34$; $P < 0.01$), whereas CH_4 and N_2O were only associated with soil moisture. For cumulative gas production, the only effect of dietary treatment was upon CO_2 and gross CO_2 equivalent (CO_2e) at the College Station site ($P \leq 0.001$), with both demonstrating a linear reduction with increased dietary QT inclusion. At both locations, there were relationships for period with CO_2, CH_4, N_2O, CO_2e, and N emission factors ($P \leq 0.07$), with period 2 having greater gas production relative to period 1. Variance partitioning indicated that animal nested within treatment and period had the largest effect upon fecal gas flux, signifying that dietary treatment and seasonal period likely influenced animal digestive and metabolic parameters to some degree. Within certain environments, it is possible that QT supplementation could reduce fecal gas emissions.

4.2. Introduction

Ruminant species are required for efficient production of human-edible protein to help meet 2050 global food demand estimates (FAO, 2017; CAST, 1999). However, gaseous byproducts from ruminant production systems such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) are deleterious to the environment (Tedeschi and Fox, 2018). Globally, manure on pasture accounted for 15% of global agricultural

non-CO_2 emissions in 2010 (Smith et al., 2014; Tubiello et al., 2014). It is estimated that CH_4 and N_2O from managed waste and grazed lands, and CO_2 from grazed lands account for roughly 54% of emissions from the livestock sector within the U.S.A., 80% of which is associated with beef and dairy cattle (USDA, 2016).

Feed additives improve ruminant growth efficiency and emission status, but societal concerns on the use of antimicrobials in food animal production have encouraged the pursuit of natural alternatives (Patra and Saxena, 2010). Condensed tannins (CT) are a plausible alternative rumen modulator that have been extensively studied based on their reactivity when in proximity to proteins, carbohydrates, microbes, and enzymes (Haslam, 1989). Within ruminant nutrition, CT are recognized for reducing feed intake and fiber digestibility (Waghorn and McNabb, 2003) but can potentially improve nutrient use efficiency and environmental footprint through protein sparing, CH_4 mitigation, and shifting N excretion to feces (Waghorn et al., 1987). However, CT supplementation effect upon fecal gas emissions is relatively unknown. The objective of this study was to determine the effect of differing rates of quebracho (*Schinopsis balansae*) CT added to a roughage-based diet upon fecal gas flux at two latitudes during two seasonal periods.

4.3. Materials and Methods

The animals used in this experiment were registered and cared for according to guidelines approved by the Institutional Animal Care and Use Committee (AUP 2017-0306) at Texas A&M University.

4.3.1. Study Sites and Experimental Design

The experimental sites were located at Texas A&M University Nutrition and Physiology Center in College Station, TX, USA (30° 36' N, 96° 20' W; altitude 112 m) and Texas A&M AgriLife Research in Stephenville, TX, USA (32°13' N, 98°12’ W; altitude of 388 m). The College Station site consisted of Boonville, Rader, and Zulch fine sandy loam soil series with a mean annual temperature of 19.4° C, mean annual precipitation of 914 to 1117 mm, and a frost-free period of 260 to 290 d (Web Soil Survey, 2017; https://websoilsurvey.nrcs.usda.gov; accessed 13 March 2019). At the Stephenville location the soil series was composed of Windthorst fine sandy loam with a mean annual temperature of 17.6° C, mean annual precipitation of 737 to 1016 mm, and a frost-free period of 210 to 240 d (Web Soil Survey, 2017; https://websoilsurvey.nrcs.usda.gov; accessed 13 March 2019). Previous management at both sites consisted of maintenance clipping with no grazing or cultivation within the preceding four years. To investigate seasonal effects, fecal gas fluxes were determined at each location during two periods corresponding with winter (P1; 1 January 2018 – 16 February 2018) and spring (P2; 9 April 2018 – 18 May 2018). Meteorological data were collected from the local municipal airports with instantaneous soil temperature and volumetric water content (VWC) for the duration of gas collections using EC-20 soil moisture and RT-1 soil temperature sensors connected to Em5b data loggers (Decagon Devices, Inc., Pullman, WA).

4.3.2. Animal Feeding and Feces Sampling

Twelve crossbred beef steers (236 ± 16 kg) were utilized to determine the effect of quebracho (*Schinopsis balansae*) CT extract (QT; SILVATEAM, San Michele, Mondovi, Italy) at 0, 1.5, 3, and 4.5% of dry matter (DM; QT_0, $QT_{1.5}$, QT_3, and $QT_{4.5}$) upon fecal gas flux. The Large Ruminant Nutrition System (http://www.nutritionmodels.com/lrns.html; accessed 3 November 2017; Tedeschi and Fox, 2018) was used to formulate a roughage-based total mixed ration (Table A-16) to meet maintenance requirements with the addition of QT serving as dietary treatments. Within each period three steers, each considered replicated experimental units, were randomly assigned to each dietary treatment. To better encompass gas flux variation due to the animal effect, all animals received different treatments in each period.

Animals were housed outside, by treatment, within concrete pens (9.1 × 12.2 m) fitted with Calan-gate feeders (American Calan, Northwood, NH), with feeding occurring once daily at 0800 h and free access to water. The basal diet was offered at 1.70% of shrunk body weight, DM basis, with pre-weighed QT being hand mixed into individual animal feed prior to provision. Animals were adapted to diets for 12 d before collection of fresh feces over two days.

Fresh feces were collected by visually observing animals and immediately collecting dung following a witnessed defecation event. Individual animal feces were placed within storage bags and promptly stored at 4°C. Following the two-day collection period, feces for an individual animal were homogenized and pre-weighed for placement

within chamber collars the following day. Excess dung was prepared for chemical analyses by drying at 55° C for 72 h or until weight loss ceased. Samples of the basal diet were collected daily for four days before fecal collections and dried at 55° C for 48 h then ground to pass through a 2-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). A 50-g subsample of feed and feces were shipped to Cumberland Valley Analytical Services (Waynesboro, PA) for chemical analysis of dry matter (DM; Goering and Van Soest, 1970), neutral detergent fiber with addition of amylase and sodium sulfite (aNDF; Van Soest et al., 1991), acid detergent fiber (ADF; Method# 973.18; AOAC, 2000), lignin, crude protein (CP; Method# 990.03; AOAC, 2000) (Leco FP-528 Nitrogen Combustion Analyzer, Leco Corporation, St. Joseph, MO), soluble CP (Krishnamoorthy et al., 1982), non-fibrous carbohydrates (NFC), total digestible nutrients (TDN), net energy (NE), fat (Method 2003.05; AOAC, 2006), starch (Hall, 2009), and a mineral panel (Method# 985.01; AOAC, 2000) (Perkin Elmer 5300 DV ICP, Perkin Elmer, Shelton, CT) (Table A-17).

4.3.3. Gas Collection

Gas was collected using vented static chambers according to the protocols of Parkin and Venterea (2010) and de Klein and Harvey (2012). Chambers consisted of two parts, soil collar (for containing fecal pat) and chamber cap (Figure B-3). Polyvinyl chloride pipe was used to construct the chambers with the collar having an area of 324 cm^2 (Parkin and Venterea, 2010) providing a chamber area-to-perimeter ratio of 19.7 cm (Rochette and Eriksen-Hamel, 2008). The chamber cap was fitted with a vent tube and

sampling port that housed a butyl septum, as well as a rubber gasket and outer rubber seal to mitigate gas leakage. Two weeks before initial gas collection, soil collars were permanently placed for the duration of the trial with 10 cm inserted into the soil and 10 cm protruding above the soil surface; chamber caps were placed on collars only for the duration of gas sampling on an individual sampling day.

At each location, 28 collars were placed in two rows of 14 (12 feces and two background soil flux) with collars within a row being spaced 50 cm apart and rows being separated by 2-m. Upon completion of P1, chamber collars were relocated to an adjacent area maintaining a similar soil profile and management. Within each row, on Day 0, 750 g of wet feces from an individual animal was placed on the soil surface within a single collar according to predetermined random assignment. Each treatment was replicated in triplicate within a row, whereas each row provided replication of individual animals. Daily collections were made by row utilizing the same progression within and between rows.

Gas fluxes were collected on Days 0, 1, 2, 4, 6, 8, 10, 12, 15, 18, 22, 25, 29, 32, 36, and 39-d after fecal application with fecal placement and gas collections occurring on the same calendar day at both locations. Sampling frequency was designed to not exceed four days between collections to maintain accurate estimation of cumulative gas fluxes (Parkin, 2008; Barton et al., 2015); however, inclement weather on Day 8 of P1 at both locations and Days 12 and 25 during P2 at Stephenville precluded data collection. All gas flux collections occurred between 0900 and 1100 h with sampling occurring at 0, 12, 24, and 36 min deployment times following chamber cap placement. Gas samples

were collected using airtight glass syringes fitted with a two-way stopcock, a 20-mL gas sample from each chamber during each deployment time was injected into a pre-evacuated 10-mL exetainer vial (Labco Limited, Lampeter, Ceredigion, UK). Following collection, gas samples were stored at 4°C until chemical analyses were conducted using a gas chromatograph (Varian 450, Palo Alto, CA) equipped with electron capture, flame ionization, and thermal conductivity detectors for determination of CO_2, CH_4, and N_2O (Holland et al., 1999).

Daily mass-based fluxes of CO_2, CH_4, and N_2O were determined from the slope of the mass concentration ($\mu g/cm^3$) vs deployment time (h) curve (Parkin and Venterea, 2010). Linear regression was used to calculate the slope when the slope was constant over time, while quadratic regression was utilized when the concentration fluctuated over time using the first derivative at time zero for flux calculation (Wagner et al., 1997). The minimum detectable limit was determined for each gas. When concentrations fell below the minimum detectable limit of the gas chromatograph for individual gases a flux of zero was assumed. The calculated hourly gas fluxes were extrapolated to represent daily emissions. Cumulative gas production was calculated as the sum of all collections using linear interpolation to estimate fluxes on non-collection days. Calculation of CO_2 equivalent emissions (CO_2e) was performed using the 100-year global warming potentials for CH_4 of 28 and N_2O of 265, by multiplying the raw gas value by the corresponding global warming potential (IPCC, 2014). Emission factors for N_2O were calculated as the cumulative N_2O-N emitted as a proportion of the N within feces (Sordi et al., 2014). Total CO_2e was defined as the sum of CO_2e for CH_4 and N_2O

and gross CO_2e as the sum of CO_2 and total CO_2e. Cumulative gas production, total CO_2 equivalent emissions (CO_2e), and gross CO_2e were evaluated per unit of DM incubated, whereas daily gas fluxes were evaluated without correcting for DM.

4.3.4. Statistical Analyses

All statistical procedures were performed using SAS software (SAS Institute Inc., Cary, NC). High levels of collinearity between soil parameters and location occurred within a period. Therefore, data were analyzed by location to determine the effect of dietary treatment and period within a location (College Station or Stephenville). Daily and cumulative flux data were analyzed using PROC MIXED with animal nested within treatment and period as random factors. Analysis of daily flux was performed by collection day to adjust for missed observations. Residuals for daily and cumulative fluxes exhibited unequal variances; heteroscedasticity was accounted for by adjusting the denominator degrees of freedom using the SATTERTHWAITE option and estimating treatment variances separately using the GROUP option within the REPEATED statement. Mean comparisons were performed using LSMeans for all significant effects ($P \leq 0.05$) and tendencies assumed at ($P \leq 0.10$). Polynomial orthogonal contrasts were performed for cumulative fluxes to determine linear, quadratic, and cubic effects of CT inclusion in the diet. Using PROC CORR, Spearman correlation coefficients were determined for fecal composition and cumulative fluxes, as well as environmental influence on daily gas fluxes.

4.4. Results

4.4.1. Daily Gas Flux

Overall trends for daily soil temperature were comparable between locations. However, location differences within the period were observed for soil VWC. The VWC at College Station was variable in P1 relative to Stephenville. In P2, College Station had greater VWC than Stephenville (Figures B-4 and 5). There was a moderate relationship between location and soil moisture (r = 0.33; P < 0.01; Table A-18) with a weak negative correlation with soil temperature (r = -0.13; P < 0.01). Period was weakly correlated with VWC (r = 0.21; P < 0.01) but highly correlated with soil temperature (r = 0.84; P < 0.01). Both soil temperature and VWC were moderately correlated with daily CO_2 production (r = 0.34; P < 0.01). However, soil temperature did not appear to be related to CH_4 or N_2O production, but VWC was positively correlated with both gases (CH4: r = 0.23; P < 0.05 and N20: r = 0.12; P < 0.05).

4.4.1.1. College Station

Daily CO_2 flux varied as a function of dietary treatment × period interactions (P < 0.05) for Days 2, 4, 12, and 15 (Figure B-6). On days 2, 4 and 15, P2 (spring) demonstrated greater CO_2 flux with gas production decreasing as QT rate increased, whereas no treatment effects were found during P1 (winter). In contrast, on Day 12, P1 exhibited larger CO_2 flux with $QT_{1.5}$ having elevated gas production relative to other treatments, but no treatment differences were evident during P2. Period (season) effects were present (P < 0.001) on Days 1, 6, 10, 29, 32, and 36 with P2 averaging 55% more

daily CO_2 production. A treatment effect was only evident on Day 6 ($P < 0.001$) with QT_0 and $QT_{1.5}$ having greater CO_2 fluxes than the other treatments. Fecal CH_4 varied as a function of dietary treatment × period interactions ($P < 0.05$) on Day 2 and 4 (Figure B-7). On Day 2, during P2, $QT_{4.5}$ had the largest flux and QT_3 was intermediate. In contrast, on Day 4, QT_0 within P1 had the greatest flux with QT_3 of P2 having the least gas production. A period effect ($P < 0.001$) was present for Days 1 and 6 with P2 having greater fluxes on Day 1 and P1 having increased gas production on Day 6. Subsequently, all fluxes decreased below the minimum detectable limit. The flux of N_2O was negligible in P1 and exhibited three peaks during P2 with period effects present on Days 0, 1, 4, and 10 (Figure B-8).

4.4.1.2. Stephenville

Dietary treatment × period effects ($P < 0.05$) were detected for CO_2 on Days 1, 2, and 15 (Figure B-9). Days 1 and 2 had increased gas production during P2 with a treatment separation evident between $QT_{1.5}$ and QT_3, as QT_0 and $QT_{1.5}$ had lower fluxes on Day 1 and the inverse occurring on Day 2, but no treatment differences were present for P1 on either day. However, on Day 15, P1 exhibited the highest flux levels with $QT_{1.5}$ within P1 having the greatest gas production. There were period effects on Days 0, 4, 6, 10, 18, 22, 29, 32, 36, and 39 with P2 having increased gas production on all days compared to P1, except Day 18 that demonstrated a greater flux during P1 relative to P2. For all measurement days, on average, daily CO_2 production within P2 increased 65% compared to P1. On Day 12, P1 was not collected; however, a treatment effect ($P =$

0.01) was present for P2 with $QT_{4.5}$ having much lower gas production. Methane exhibited period effects ($P < 0.001$) on Days 1, 2, and 4 as P2 demonstrated an 82% increase in gas production compared to P1, on average (Figure B-10). Period effects ($P < 0.05$) were present for N_2O on Days 2 and 32 with P1 having increased gas flux on Day 2, whereas on Day 32 fluxes were only observed during P2 (Figure B-11).

4.4.2. Cumulative Gas Flux

4.4.2.1. College Station

Cumulative CO_2 production demonstrated dietary treatment and period effects ($P = 0.014$ and $P < 0.001$, respectively; Table A-19). A significant linear trend was present for treatment ($P < 0.001$) as CO_2 gas flux decreased with increased dietary QT inclusion, whereas P2 exhibited 53% greater cumulative flux than P1. Cumulative CH_4 flux differed between periods ($P = 0.025$) where P2 had an 87% increase in flux compared to P1. A similar trend was evident for N_2O with a tendency ($P = 0.079$) for increased N_2O production during P2. Total CO_2e demonstrated a period effect ($P = 0.01$) with 91% less flux occurring within P1. On average, all QT treatments exhibited numerically lower fluxes than the control. Treatment and period gross CO_2e differed ($P \leq 0.001$) with the same trends as those demonstrated for CO_2 production. Gross CO_2e was 54% greater during P2 with a linear effect ($P < 0.001$) for decreased CO_2e with increased dietary QT provision. There was a period tendency ($P = 0.054$) for total N emission factor, with P2 losing roughly twice as much N in the form of N_2O relative to P1. On average, 38% less total N was emitted as N_2O within QT treatments relative to the control. Similarly,

emission factors for soluble N were reduced 149% in P1 ($P = 0.029$) with QT inclusion decreasing soluble N loss 35% on average.

4.4.2.2. Stephenville

There were no interactions or treatment effects for cumulative gas flux at Stephenville ($P > 0.616$; Table A-20). Production of CO_2 was 450% greater in P2 relative to P1 ($P < 0.001$) with all treatments sharing similar cumulative flux estimates. Similarly, CH_4 and N_2O demonstrated greater gas production within P2 ($P < 0.001$ and $P = 0.029$), increasing 240% for both gases on average. Total CO_2e production was increased 254% during P2 with $QT_{1.5}$ having a reduced flux estimate compared to all other treatments. A large period difference was present for gross CO_2e ($P < 0.001$) as emissions within P2 were 443% greater than P1. Emission factors for total and soluble N were substantially greater during P2 ($P = 0.026$ and $P = 0.018$) where N lost as N_2O increased 259 and 426% for total and soluble N, respectively.

4.5. Discussion

4.5.1. Daily Gas Flux

Within the current trial, we observed large variation in daily gas fluxes across periods. Based upon the correlation analysis, soil moisture and temperature were the major determinants of daily fecal gas flux, given the parameters measured in this study. Soil gas fluxes are largely affected by temporal variability as environmental factors, such as soil moisture and temperature (Rochette et al., 1991), are the major determinants of

soil microbial growth and activity (Pietikäinen et al., 2005). A reduction in soil moisture results in decreased total microbial biomass with minor effects on community compositions (Ren et al., 2018); whereas, optimal growth temperature for soil bacteria and fungi range from approximately 25 to 30°C with temperatures outside this range resulting in reduced microbial activity (Pietikäinen et al., 2005). Although not fully assessed, cattle feces encompass a dynamic consortium of microflora that is largely influenced by the host animals diet (Jost et al., 2013). Therefore, fecal gas fluxes should respond to temporal variation similar to soil gas fluxes due to analogous microbial processes taking place (Waldrip et al., 2016).

Based upon our data, it appears that soil moisture and temperature are associated with daily fecal gas flux to varying degrees for individual gases, with location and period differences being greatly influenced by local meteorological conditions. Across locations, soil temperatures remained similar with differences in soil moisture being more pronounced. The period effects observed within the current study are largely a result of soil temperature, which is assumed to correspond with fecal material, being below the optimal range for microflora activity during P1. Although soil temperatures during P1 were below the optimal range, microbial processes were still occurring to some degree as the soil temperatures did not decrease below the assumed minimum temperature for microbial activity, -6° C (Pietikäinen et al., 2005). Soil temperatures during P2 ranged from 14 - 28° C, nearing the optimal temperatures for bacterial and fungal growth during the latter part of the period. It is expected that gas fluxes would increase as the soil temperature nears the optimal range for microflora; however, this

response was not observed. Lack of response to increasing temperatures is likely due to decreased moisture content reducing substrate availability (Ren et al., 2018) and created an aerobic environment that is not conducive CH_4 and N_2O production (Nazaries et al., 2013; Waldrip et al., 2016). Although precipitation events were observed during both periods, feces tend to form a crust during the drying process that reduces moisture uptake by the fecal pat and results in moisture being localized to the soil-feces interface. Therefore, soil temperature likely corresponds to fecal temperature to some degree, but VWC does not provide a good indication of the entire fecal pat's moisture content.

4.5.2. Cumulative Gas Flux

Comparatively, cumulative gas production during P1 was substantially larger at the College Station location as fluxes ranged from 38 to 80% greater relative to Stephenville, likely a result of greater soil temperature as both locations had similar VWC. In contrast, locations were more similar during P2 with College Station maintaining 27 to 30% more gas production for all gases with the exception of CH_4 and total CO_2e. Location differences during P2 are more indicative of the difference in precipitation received since temperatures were comparable at both locations. The discrepancies in cumulative flux estimates across sites are primarily attributable to differences in environmental parameters discussed previously, but soil properties between locations could have exacerbated the environmental differences. The Windthorst soil series at the Stephenville location has greater drainage and permeability than the Zulch soil series of College Station. Therefore, it is assumed Stephenville would

have a shorter period of soil wetness following a precipitation event, decreasing moisture absorbance by the feces and deleterious to an anaerobic environment. In the current study, gas fluxes indicate that drainage class of these soils likely correspond with gas production to an extent; however, it has been shown that soil drainage class does not always coincide with gas fluxes due to intrinsic soil properties of the upper profile that can result in longer periods of soil wetness (van der Weerden et al., 2011).

The nutritional content of feces did not have an apparent effect on fecal gas flux (Table A-21); however, correlation coefficients for pooled data do not agree with previous data from our research group (preliminary data) utilizing feed-through QT. Within the current trial, total N was negatively associated with CO_2 and CH_4, r = -0.23 and -0.38 respectively, with no effect on N_2O. Soluble N and NFC demonstrated moderate negative correlations with all gases. This is in contrast to Jost et al. (2013) who noted increased CO_2 production with increased N and carbohydrates, whereas CH_4 yield increases with greater fermentable substrate (Hashimoto et al., 1981). The negative correlations observed within this trial appear to be due to the effect of QT upon metabolic parameters and QT efficacy following exposure to the digestive system that ultimately influenced manure composition. Treatments $QT_{1.5}$ and QT_3 had the greatest levels of total N but demonstrated numerically lower CO_2 and CH_4 production, with the inverse occurring for QT_0 and $QT_{4.5}$. Therefore, within the current trial, it likely is not the absolute amount of nutritional entities that determine gas flux but rather the microbially available nutrients that is a byproduct of QT binding capacity following

exposure to the gastrointestinal tract, as well as the resulting effect on the physical composition of feces that influence microbial activity.

Fibrous constituents only demonstrated a slight positive association with CO_2, r = 0.21 and 0.25 for NDF and ADF, respectively. There was a moderate association of CO_2 with CH_4 and N_2O, r = 0.46 and 0.33, respectively. As CO_2 production is the primary byproduct of fermentation, increased CH_4 tends to follow spikes in CO_2 production due to greater availability of methanogenic precursors (Hashimoto et al., 1981). Accordingly, CO_2 and N_2O frequently follow similar trends as organic carbon and N are typically linked within substrates (Xu and Zhou, 2008). Since CH_4 had substantially larger fluxes than N_2O, total CO_2e was very strongly related to CH_4 (r = 0.98; $P < 0.01$). Similarly for gross CO_2e, the flux of CO_2 accounted for more than 99% of the variation. The strong relations between individual gases and CO_2e parallels previous data from our research group (preliminary data) investigating the effect of QT on fecal gas flux.

The emission factors observed within our trial are greatly reduced relative to other published estimates, ranging from 0.01 – 0.15% (van der Weerden et al., 2011; Sordi et al., 2014). Sordi et al. (2014) collected gases over 90 d using three deployment times with measurement intervals being 2-to-3 d for 2 weeks and 40 d for the last two samplings. Similarly, van der Weerden et al. (2011) acquired samples over an undisclosed period of time that exceeded eight weeks and utilized three deployment times biweekly for one month followed by two deployment times once per week after that. Both studies utilized linear models to determine gas fluxes that can result in biased

estimates and lacks the robustness of non-linear methods when less than 4 deployment times are utilized (de Klein and Harvey, 2012). As well, the sampling frequencies used increased the inherent error associated with N_2O estimates as fluxes sampled at 3-to-7 d have demonstrated deviations of -18 to + 24% of the actual cumulative N_2O emissions, whereas 14-d intervals ranged from -43 to +64% (Parkin, 2008). Therefore, precise estimation of N_2O fluxes require a sampling interval ranging from 1-to-4 d, but can be expanded to 6-d and retain some degree of precision (Parkin, 2008; Barton et al., 2015). The differences in the sampling procedure, dietary treatments, and analytics could explain some of the discrepancies between our results and previous findings, but the length of sampling likely imparted the largest effect.

The variance partitioning for individual gases (Table A-22) denotes that animal nested within treatment and period accounted for 34, 81, 74, and 34% of the variance for CO_2, CH_4, total CO_2e, and gross CO_2e, respectively. Since animal accounted for such a large proportion of the total variance, differences in the effect of fecal nutrients on gas flux within this study appear to be due to increased animal variation. The high degree of animal variation observed is likely attributable to individual animal response to QT in the diet and environmental conditions during the period, resulting in altered digestive kinetics and metabolic functions.

4.6. Conclusion

Feeding QT influenced fecal gas emissions depending on environmental conditions. Soil moisture and temperature seemed to have the greatest influence on

potential gas emissions. As moisture and temperatures increased from P1 to P2 a large increase in fecal gas fluxes were observed, presumably due to environmental conditions conducive to microbial activity. Soil type appears to also play a key role due to the influence of soil drainage upon soil moisture in the upper profile. Congruent with independent data from our research group (preliminary data) CO_2 was the predominant gas produced, accounting for the vast majority of gross CO_2e. The only treatment effects observed were within the College Station study site, as CO_2 and gross CO_2e were reduced at the two highest levels of QT inclusion.

There was no treatment × period interaction for either location, but all gases within each location demonstrated a period effect. At both locations, P2 had substantially higher gas flux, with a much larger increase from P1 to P2 at the Stephenville location. Even so, in terms of absolute emissions, the College Station site had higher gas fluxes than Stephenville, chiefly driven by temperature differences between locations. These data demonstrate that within warmer environments QT supplementation could potentially reduce more fecal gross greenhouse-gas emissions of CO_2, CH_4, and N_2O than in cooler regions. At the very least, fecal decomposition rates, if not total decomposition, will slow with lower temperatures. However, as we did not investigate urinary emissions, which represents the greatest proportion of N lost as N_2O-N, how QT supplementation may affect urinary N emission factor through possible alteration of N form is unclear and merits further study.

Additionally, future research determining excreta emissions dynamics should utilize increased animal numbers, especially when evaluating potential mitigation

techniques, due to animal accounting for a considerable portion of the total variance. The effect of the animal appears to be a culmination of individual metabolic response to QT that influences manure composition, as well as lower-tract microflora composition that determines the prevalent microflora within feces. These differences are driven by genetics and environment (e.g., individual animal health, feed conditioning or rumen microorganism genomics) to varying degrees and warrants investigation of the dynamic endogenous relationship.

Based upon our results, the investigation of greenhouse-gas emissions from animal agriculture should include a proxy that represents all greenhouse gas emissions, not solely CH_4 and N_2O, as results could be misconstrued and not represent actual system efficiency. The utilization and integration of excretory, metabolism, and respirometry data should define production efficiency as human-edible nutrient on a CO_2e per unit nutrient basis, enabling the advancement of more efficient production practices.

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5. INFLUENCE OF QUEBRACHO TANNIN EXTRACT FED AT DIFFERING RATES WITHIN A HIGH-ROUGHAGE DIET ON DIGESTIBILITY, NITROGEN BALANCE, AND PARTITIONING OF ENERGY

5.1. Overview

Gaseous byproducts from ruminant production, such as methane (CH_4) and nitrous oxide (N_2O), reduce energy efficiency and can be detrimental to the environment. Antibiotics improve feed efficiency by decreasing morbidity and changing rumen dynamics. However, issues regarding antimicrobial resistance and chemical residues have elicited a search for natural rumen modulators. Condensed tannins (CT) are an alternative feed additive that could improve animal and system-level efficiency due to enhanced protein efficiency and reduced CH_4. We evaluated how quebracho tannin (QT) extract fed at 0, 1.5, 3, and 4.5% of DM, within a roughage-based diet affected apparent digestibility of DM, OM, and fibrous fractions, and N retention and energy partitioning. Utilizing a Latin rectangle design with eight animals and four periods, whole-animal CO_2, O_2, and CH_4 production and uptake were measured and total feces and urine were collected over a 48-h period using open-circuit, indirect calorimetry respiration chambers. Following removal from chambers, rumen inoculum was collected for ruminal parameter determination. Dry matter and gross energy intake were influenced by the level of QT inclusion ($P \leq 0.036$). Digestibility of DM, OM, and N was reduced with QT inclusion ($P < 0.001$), but fiber digestibility was not greatly impacted ($P > 0.123$). Quebracho tannins altered N excretion route, average fecal N-to-

total N ratio excreted increased 14%, and fecal N-to-urinary N ratio increased 38% ($P < 0.001$); however, N retention was not affected. Increased fecal energy with QT provision resulted in reduced DE/ kg DMI ($P = 0.024$). There were no differences in urinary energy ($P = 0.491$), but CH_4 energy decreased ($P = 0.007$) as QT inclusion increased. Metabolizable energy was not affected by QT inclusion and the conversion efficiency of DE to ME did not differ. Heat energy decreased ($P = 0.013$) with increased QT inclusion rate, but there was no difference in retained energy. There were no differences for retained energy or N per CO_2e produced ($P = 0.774$ and 0.962, respectively), but improved efficiency for energy retention was apparent for QT_3. We conclude that QT provided up to 4.5% of DMI does not affect N and energy retention within the current setting. Feeding QT reduced energy losses in the form of CH_4 and heat, but the route of energy loss appears to be influenced by the rate of QT inclusion.

5.2. Introduction

Ruminant species are a vital source of human-edible protein and essential nutrients due to their capacity to upgrade low-quality feedstuffs (CAST, 1999) but gaseous byproducts, such as ammonia (NH_3), carbon dioxide, (CO_2), and methane (CH_4) produced during anaerobic microbial fermentation have a perceived negative impact in the environment by possibly augmenting global warming. Besides aiding in maintaining ruminal homeostasis (McAllister and Newbold, 2008), gaseous byproducts also reduce energetic efficiency. Within the agricultural sector, CH_4 and nitrous oxide (N_2O) are the predominant greenhouse gases (GHG), with enteric fermentation (~40%) and manure on

pasture (~15%) representing 47 to 56% of total global agricultural non-CO_2 emissions in 2010 (Smith et al., 2014; Tubiello et al., 2014). However, on a CO_2 equivalent emissions basis (CO_2e) 72 to 76% of total global GHG emissions are from CO_2, of which agriculture, forestry, and other land uses account for approximately 14% (IPCC, 2014).

Feed-grade antimicrobials decrease ruminant morbidity and alter rumen dynamics, promoting growth efficiency (Yang and Russell, 1993; Guan et al., 2006). However, consumer concerns about food safety have prompted the pursuit of natural rumen modulators (Patra and Saxena, 2010). Because condensed tannins (CT), a diverse group of naturally occurring secondary metabolites, display reactivity when in proximity to proteins and carbohydrates (Haslam, 1989) they could potentially serve as a ruminant feed additive. Utilization of CT commonly results in reduced DMI and ruminal digestibility due to astringency, slower passage rate, and microbial inhibition (Landau et al., 2000; Frutos et al., 2004). However, CT have displayed potential for improved nutrient and energy efficiency (Piñeiro-Vázquez et al., 2017). The objective of this study was to determine the effect of feeding quebracho CT extract at four inclusion rates within a roughage-based diet on apparent digestibility of fibrous fractions, nitrogen (N) retention, energy partitioning, and ruminal parameters.

5.3. Materials and Methods

The animals used in this experiment were registered and cared for according to guidelines approved by the Institutional Animal Care and Use Committee (AUP 2017-0306) at Texas A&M University.

5.3.1. Experimental Design, Equipment, and Data Collection

A 4 x 8 Latin rectangle design with four periods and 8 British crossbred steers (236 ± 16 kg BW) were used to determine the effects of quebracho (*Schinopsis balansae*) CT extract (QT; SILVATEAM, San Michele, Mondovi, Italy) at 0, 1.5, 3, and 4.5% of feed DM (QT_0, $QT_{1.5}$, QT_3, and $QT_{4.5}$), so that each treatment was replicated by two animals within each period. The Large Ruminant Nutrition System (http://www.nutritionmodels.com/lrns.html; accessed 24 April 2018; Tedeschi and Fox, 2018) was used to formulate a roughage-based total mixed ration (Table A-23), with the addition of QT serving as dietary treatments. Animals were provided the basal diet at 1.70% of BW, DM basis, to meet maintenance requirements with pre-weighed QT hand-mixed into individual animal feed before provision. Animals were housed outside within pens (9 × 12 m) fitted with Calan-gate feeders (American Calan, Northwood, NH). Feeding occurred once daily at 0800 and animals were provided free access to water. For each experimental period, dietary adaptation of each animal spanned 12 d followed by relocation to open circuit, indirect calorimetry respiration chambers for measurement of gas exchange and total feces and urine over 48 h. Because only two respiration chambers were available, data from two steers were collected at one time. Upon completion of a 48-h measurement period, chambers were recalibrated, and a new pair of animals entered the chambers. Therefore, gas exchange and excretory data collection spanned 8 d within each period.

5.3.2. Temperature and Humidity

For all periods, on Day 12 for a pair of steers, 18-h shrunk BW (SBW) were recorded prior to entering a predetermined single-stall open-circuit, respiration calorimetry chamber with a volume of 11.5 m^3. Chambers were maintained at thermoneutral conditions (18 ± 0.55 °C; 55 ± 1.2% RH), as determined by the temperature-humidity index, and corresponding to 18.9 °C based upon the current effective temperature index (Tedeschi and Fox, 2018). Thermoneutral environments were maintained using a line voltage thermostat (Ranco Enterprises, Inc., Model# ETC-111000-000) and dehumidifier (Hisense USA, Model# DH-70K1SDLE) with environmental conditions monitored using digital HOBO temperature and humidity data loggers (Onset Computer Corporation, Model# UX100- 003). Water intake and activity within chambers were monitored using a water meter (Neptune Technology Group, Inc., Model# T10-DR-075-G-F) and security cameras (FLIR Lorex Inc., Model# LBV1511W). In addition, each chamber was equipped with a metabolism stand to allow the collection of total urine and fecal output. Following the 48-h period within chambers, rumen inoculum was collected, and animals were returned to Calan-gate pens to begin 12-d adaptation to subsequent diets for the next experimental period.

5.3.3. Open Circuit, Indirect Calorimetry Respiration Chambers

Respiration chambers were configured in a pull-type arrangement using a mass flow system (Flowkit model FK-500; Sable System Int., Henderson, NV), creating a slight negative pressure within chambers. Within this system ambient air (baseline) and

air from each chamber was sampled via a multiplexer (Respirometry Multiplexer V 2.0, Sable System Int., Henderson, NV) and a FC-1B O_2 analyzer, CA-2A CO_2 analyzer, and MA-10 CH_4 analyzer (Sable System Int., Henderson, NV) rotating every 4 min. Before initiating gas sampling for a pair of animals, SBW, dietary energy density, and volume of the calorimetry chambers were used to calculate the time required for gas concentrations to stabilize and flow rate needed to maintain chamber CO_2 concentrations between 0.35 and 0.37%. The assumed gas concentrations of baseline ambient air (O_2 = 20.95%, CO_2 = 0.04%, and CH_4 = 0.00%) were used to calibrate O_2, CO_2, and CH_4 analyzers using known gases, N (99.999% N_2; zeroing gas) and SPAN (19.4, 1.1, and 0.1% O_2, CO_2, and CH_4, respectively) before each set of steers entered for data collection. The measured gas was scrubbed of water vapor using fresh drierite desiccant (Hammond Drierite Co Ltd, Xenia, OH) for each 48-h collection, and the rate of O_2, CO_2, and CH_4 uptake and production (VO2, VCO2, and VCH4; L/min) were determined (Lighton, 2008). Prior to each period, the sealing condition of each chamber was checked using a manometer. Once adequate chamber sealing was achieved, chamber measurements were validated using gravimetric N injection technique (Cooper et al., 1991) to perform oxygen dilutions, where expected (20.95% × volume of N) and observed VO2 uptake were verified with recovery ranging from 99 to 101%. All flow rates were adjusted to a dry-gas basis by correcting for water vapor concentration calculated from temperature and RH (Lighton, 2008).

5.3.4. Sample Collection, Preservation, and Analyses

Basal diet batch samples (250 g) were collected daily for the last 10 d of each period. A 50-g feed composite from each period was shipped to Cumberland Valley Analytical Services (Waynesboro, PA) for chemical analysis of DM (Goering and Van Soest, 1970), NDF with addition of amylase and sodium sulfite (aNDF; Van Soest et al., 1991), ADF (Method# 973.18; AOAC, 2000), ADF lignin using a modified version of the Goering and Van Soest (1970) method, CP (Method# 990.03;AOAC, 2000) (Leco FP-528 Nitrogen Combustion Analyzer, Leco Corporation, St. Joseph, MO), soluble CP (Krishnamoorthy et al., 1982), fat (Method# 2003.05; AOAC, 2006), starch (Hall, 2009), sugar (Dubois et al., 1956), a complete mineral panel (Method# 985.01; AOAC, 2000) using a Perkin Elmer 5300 DV ICP (Perkin Elmer, Shelton, CT), and calculated Non-fibrous carbohydrates (NFC), TDN, and NE.

During the duration of this experiment, no feed refusals or orts were observed. Following removal of animals from chambers, feces were weighed, homogenized, subsampled and stored in a –20 °C freezer. Fecal samples were dried at 55 °C for 72 h or until weight loss ceased, then ground to pass through a 2-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ), and analyzed for DM, OM, CP, aNDF, ADF, and GE.

Total urine collection, acidification, and removal from chambers were accomplished using the method discussed by Crossland et al. (2018); where total urine was weighed, and two 100-mL subsamples were stored at -20 °C. Total urinary N

analysis was performed by Servi-Tech laboratories (Amarillo, TX) using the Dumas combustion method (Method# 990.03; AOAC, 2000).

Upon removing steers from respiration chambers, we collected 500 mL of rumen fluid using an esophageal tube connected to a vacuum pump. Rumen inoculum was filtered through 8 layers of cheesecloth and pH was immediately measured. Inoculum samples were allocated into duplicate containers for the preservation of VFA, NH_3-N, and protozoa. Preservation methods were 8 mL of inoculum and 2 mL of 25% (wt/vol) metaphosphoric acid solution for VFA analyses, 2 mL of inoculum to 8 mL of 0.1 N HCl acid solution for NH_3 analyses, and 1 mL of inoculum and 10 mL of ethanol for protozoa counts; all samples were stored at –20 °C. Concentrations of NH_3-N were determined by colorimetric methods using a commercial test and VFA using gas chromatography (Hinton et al., 1990). Protozoa counts were determined by methods described by Dehority (1984) without staining.

5.3.5. Energy Partitioning and Nitrogen Balance

Gross energy was obtained on feed, fecal, and urine samples using a bomb calorimeter (Parr adiabatic calorimeter; Parr Instruments Co., Moline, IL). Gross energy analysis of QT was also performed with the average GE value of QT being 5099.75 cal/g DM. Then, GE intake (GEI; Mcal/d) was computed by summing the GE of basal diet and QT offered, the GE of respective amendments was determined by multiplying the caloric value by kilograms offered. Fecal and urinary energy (FE and UE; Mcal/d) were calculated by multiplying the GE of respective samples by the daily output. Gaseous

energy (GASE; Mcal/d) was determined by multiplying daily CH_4 production (L/d) by the density of CH_4 at normal temperature and pressure (0.668 g/L at 20 °C and 1 atm) and the energy density of CH_4 (13.3 Mcal/kg). Heat energy (HE) was calculated according to (Brouwer, 1965): HE (Mcal/d) = (3.866 × VO2) + (1.2 × VCO2) − (0.518 × VCH4) − (1.431×Urinary N). Final values of energy partitioning were calculated as follows: DE = GEI − FE; metabolizable energy intake (MEI; Mcal/d) = DE − (UE + GASE); retained energy (RE; Mcal/d) is assumed as RE = MEI − HE, where MEI was calculated as the observed dietary ME content (Mcal/kg) multiplied by the DMI (kg/d) of diet. Carbon dioxide equivalent emissions were calculated by multiplying daily production of CH_4 by its 100-year warming potential (28), with total CO_2e being the sum of CO_2 and CO_2e from CH_4. Retained energy and nitrogen (RN)/CO_2e produced were used as measures of system efficiency.

5.3.6. Statistical Analyses

Statistical procedures were performed using PROC GLIMMIX of SAS (SAS Institute Inc., Cary, NC). All data were evaluated using a Latin rectangle design, with animal and period as random factors. To account for any carry-over effects, the previous diet was included within the initial model. However, preliminary results indicated no effect of previous diet or improvement in goodness-of-fit; therefore, the carry-over effect (i.e., previous diet) was removed from the statistical model. The significance of covariance parameter estimates was tested by applying the Wald test using the COVTEST statement. Mean comparisons were performed using the LSMEANS

statement with the adjustment proposed by Tukey-Kramer for all significant effects. Investigation of custom hypotheses was performed using contrasts constructed utilizing the Scheffé adjustment. Additionally, orthogonal polynomial contrasts were performed for all variables to determine linear, quadratic, and cubic effects of QT inclusion in the diet. Significance was established at $P \leq 0.05$ and tendencies were assumed at $P \leq 0.10$.

5.4. Results and Discussion

5.4.1. Intake, Excretion, and Digestibility

Table A-24 shows the effect of QT upon intake and excretion profiles. There was no effect of QT upon daily water intake, water as a proportion of SBW, or water intake-to-DMI ratios ($P > 0.380$). Similarly, Landau et al. (2000) did not observe an effect of CT on water consumption. During the experimental periods there were no feed refusals. However, as QT was added to the basal diet DMI and OMI were elevated ($P < 0.036$) in treatments receiving QT, but no differences in the consumption of aNDF or ADF ($P > 0.186$) were observed. Provision of CT commonly decreases DMI due to negative associations resulting from astringency and reduced passage rate (Landau et al., 2000; Frutos et al., 2004), as well as potential gastrointestinal distress at higher rates (Dawson et al., 1999; Hervás et al., 2003). Previous data from our research group (preliminary data) utilizing identical diets noted reduced intake and indicators of potential gastrointestinal distress when limit-feeding with QT. However, in the current study animals were limit-fed with all treatments being readily consumed, and no dietary aversion observed.

Daily fecal output parameters were affected ($P < 0.003$) by QT supplementation. Daily fecal DM, OM, and DM as a proportion of SBW demonstrated linear increases with greater QT inclusion ($P < 0.001$). However, there were no differences for daily fecal aNDF or ADF ($P > 0.186$). A linear increase in fecal DM production with CT inclusion rate is presumed to be a result of reduced ruminal degradation, particularly in diets containing high levels of fibrous carbohydrates (Ahnert et al., 2015; Aguerre et al., 2016). In contrast to fecal excretion, daily urine production and urine as a proportion of SBW decreased ($P \leq 0.001$) in a linear fashion with increased provision of QT. The reduction at the two highest supplementation rates was unexpected as CT in other studies have not reported an effect on urinary output volume (Ahnert et al., 2015; Aguerre et al., 2016).

The inclusion of QT affected DM and OM digestibilities ($P \leq 0.001$), with clear linear trends with a tendency for a secondary cubic effect for OMD (Table A-25). Compared to QT_0, there was no reduction in digestibility for $QT_{1.5}$ and QT_3, but $QT_{4.5}$ reduced DMD and OMD by 11%, on average. In contrast, there was no effect of QT on aNDF or ADF digestibilities ($P > 0.123$). However, trends for reduced digestibilities in a linear fashion were observed for both coefficients, with $QT_{4.5}$ reducing aNDF and ADF digestibilities approximately 9% on average. The decrease in DM and OM digestibilities agrees with Piñeiro-Vázquez et al. (2017), who observed a reduction in DM and OM digestibility with QT supplementation in heifers fed a forage-based diet. However, Piñeiro-Vázquez et al. (2017) saw a reduction when QT exceeded 1% DM, whereas in the current study, only $QT_{4.5}$ decreased digestibilities. This could be due dietary

influences, such as precipitable N and carbohydrates, or may be an effect of differences in absolute CT provided within the extracts. The increased degree of reduction for OMD compared to aNDF and ADF could denote reduced ruminal digestibility of NFC. This has been observed previously when feeding sorghum silage, as ruminal digestibility of starch decreased 7% in high-CT sorghum, but total-tract digestibility did not differ (de Oliveira et al., 2007).

Apparent N digestibility was reduced 20% on average for all QT inclusion levels ($P < 0.001$), but there was no difference in digestibility among QT treatments. The digestibility of N was reduced to a larger degree than digestibilities of DM, OM, aNDF, or ADF; this finding is consistent with previous QT research that observed greater reductions in apparent digestibility of N relative to DM and aNDF (Aguerre et al., 2016). The prominent reduction in N digestibility observed when feeding CT appears to be a product of direct complexation with soluble proteins and the accompanied lesser degradation of feedstuffs, restricting the availability of cell-wall associated proteins and decreasing the absolute amount of ruminally degradable protein. This is substantiated by Beauchemin et al. (2007) and Koenig and Beauchemin (2018), with the authors witnessing a drastic linear reduction in total-tract digestibility of ND and AD insoluble N when QT was provided in silage and concentrate-based diets. Reduced cell-wall digestibility would result in CT having a more pronounced effect upon diets with a larger concentration of B_2 and B_3 N fractions, but in diets with greater relative proportions of non-protein N and B_1 proteins, CT could potentially assist in improving N use efficiency through reduced proteolysis and increased microbial utilization of NH_3.

Provision of QT resulted in greater daily fecal N ($P < 0.001$), and $QT_{1.5}$ exhibited the greatest fecal N concentration ($P = 0.023$). A linear and cubic trend was observed for fecal N (% of N intake) with QT_0 lower than all other treatments ($P < 0.001$) and QT_3 having the least for treatments receiving QT. Alternatively, there was no difference in urinary N concentration, but daily urinary N excreted, and urinary N (% of N intake) decreased with increased QT supplementation ($P = 0.007$ and $P = 0.019$, respectively). The inclusion of QT drastically altered N excretion route, with fecal N (% of N excreted) increasing 14% and fecal N-to-urinary N ratio being 38% greater on average with QT supplementation ($P < 0.001$). However, QT did not affect N retention ($P = 0.744$). Similar to the current study, Grainger et al. (2009) witnessed increased fecal N and reduced urinary N with increasing CT levels but no difference in retained N was observed. Due to the shift of N excretion from urine to feces with QT inclusion N digestibility becomes artificially reduced, thereby making apparent digestibility unsuitable for estimating N retention when diets include CT.

In the current study, protein binding is evidenced by the large alteration in N excretion route. However, apparent N digestibility and retained N (% of N intake) is reduced relative to previous research from within our lab group (preliminary data, not shown) utilizing the same diet and treatments. Decreased N utilization could indicate lower post-ruminal disassociation of QT-protein complex, but the animal physiological stage and the previous plane of nutrition may partly explain the discrepancy between studies. As fecal N is less volatile than urinary N, shifting the excretion of N to the feces can potentially assist in reducing, or at least slowing, environmental emissions and

improve system efficiency by decreasing NH_3 and N_2O production, possibly improving N cycling within terrestrial ecosystems (Ndegwa et al., 2008; Patra and Saxena, 2011). Therefore, although N retention was not different for treatments if whole-animal emissions (i.e., enteric and excreta gas emissions) are accounted for CT might offer a method of improving system-level efficiency.

5.4.2. Open Circuit, Indirect Calorimetry

Estimates from the calorimetry assessment are shown in Table A-26. There was an effect of treatment ($P = 0.001$) for respiratory quotient (RQ) with a quadratic trend present. All treatments had relatively similar RQ values, but RQ's for QT_0 and $QT_{4.5}$ slightly exceeded 1.00. As DMI and OMI differed due to QT inclusion, GEI was different ($P = 0.002$). There was an effect of QT for FE ($P = 0.001$), FE increased linearly with greater QT rate as $QT_{4.5}$ excreted 19% more FE than QT_0. The increase in FE did not affect DE/d, but DE/ kg DMI was reduced ($P = 0.024$). Linear effects were present for DE/d and DE/kg DMI with $QT_{4.5}$ being 5 and 8% lower for both parameters relative to QT_0. In dairy cows grazing ryegrass (*Lolium* spp.), *Acacia mearnsii* CT at 1 and 1.9% of DM increased FE with increased inclusion that resulted in a 21 and 36% reduction in DE, respectively (Grainger et al., 2009). The conversion of GE-to-DE was reduced with QT inclusion ($P = 0.009$) with a linear response being present. The lower conversion efficiency of GE-to-DE is commonly observed when feeding CT. Compared to QT_0, conversion efficiency for $QT_{4.5}$ decreased by 5.5%. This is much lower than the 17% reduction in conversion efficiency seen by Piñeiro-Vázquez et al. (2017) when QT

was provided at ≥ 3% DM in a low-quality forage diet of *Pennisetum purpureum*. Similarly, DE-to-GE decreased 8 and 14% in dairy cows when supplementing *Acacia mearnsii* (Grainger et al., 2009). The large reductions observed are likely a result of GEI being reduced with CT inclusion in both trials, whereas in the present study animals were limit-fed with all treatments receiving the same proportion of fermentable nutrients.

No difference for UE was present ($P = 0.491$), although UE for QT_0 was approximately 20% greater than QT_3. The effect of CT on UE appears variable as no difference was observed in cattle receiving concentrate or warm-season forage diets (Ebert et al., 2017; Piñeiro-Vázquez et al., 2017), but reduced UE has been reported in dairy cattle and sheep provided cool-season forages (Carulla et al., 2005; Grainger et al., 2009). There was a difference in daily GASE ($P = 0.007$) with a linear reduction ($P = 0.001$) in GASE as QT inclusion increased. In the contrast analyses, a difference in GASE for QT_0 relative to all QT inclusion rates was observed ($P = 0.013$; data not shown) as the GASE of $QT_{1.5}$, QT_3, and $QT_{4.5}$ was reduced approximately 6, 6, and 17%, respectively. Previous research regarding the effect of CT on GASE is variable. Reduced GASE has been observed when supplementing QT ≥ 2% in a low-quality forage diet (Piñeiro-Vázquez et al., 2017) but no effect was evident when CT was fed within silage and concentrate-based diets (Beauchemin et al., 2007; de Oliveira et al., 2007; Ebert et al., 2017). For GASE (% of GEI), a similar trend was observed with QT_0 having the greatest GASE ($P = 0.045$), representing roughly 6.6% of GEI. The conversion of GE to GASE observed for QT_0 in this study matched the conversion factor from IPCC (2006)

inventory report (6.5 ± 1.0% for cattle grazing or fed low-quality byproducts); however, all QT containing treatments were on the lower end of the conversion factor range, 6.1, 6.1, and 5.3% for 1.5, 3, and 4.5% QT, respectively.

Daily MEI (Mcal/d) and dietary ME (Mcal/kg DMI) did not demonstrate treatment differences ($P > 0.218$). The conversion efficiency of DE-to-ME was not different with QT_0 and $QT_{1.5}$ having a ME-to-DE ratio between 0.86 and 0.87, whereas QT_3 and $QT_{4.5}$ exceeded 0.87. The ME-to-DE ratio observed within this study is greater than the 0.82 ratios utilized by NASEM (2016), but our data fit within the range of 0.82 to 0.93 suggested by Vermorel and Bickel (1980). When evaluating computations of ME from DE using the 0.82 conversion factor and the equation proposed by Galyean et al. (2016) for diets exceeding 2 Mcal/kg, estimates did not differ in precision ($r^2 = 0.92$) with Akaike's information criterion indicating slightly improved fit for the 0.82 conversion compared to the Galyean equation (-115 vs. -106). Similar to ME-to-DE ratio, there was no treatment effect for ME as a proportion of GE ($P = 0.134$) but the ME-to-GE ratio decreased linearly ($P = 0.054$) with increased CT inclusion as the conversion efficiency of $QT_{4.5}$ was 7.5% lower than QT_0.

Daily HE was affected by QT inclusion rate ($P = 0.013$) in a linear fashion ($P = 0.003$) with QT_3 HE being 5% less than QT_0. On a metabolic body weight basis, HE decreased linearly ($P = 0.019$) as QT level increased. Heat energy (% of GE) was decreased 3%, on average, for QT_3 and $QT_{4.5}$ ($P < 0.001$) with a linear trend present ($P < 0.001$). Using whole animal respirometry, a reduction in HE when feeding CT has been observed previously in sheep and goats (Carulla et al., 2005; Puchala et al., 2012). In

contrast, HE was not different for finishing steers or dairy cows when QT was added to the diet (Huyen et al., 2016; Ebert et al., 2017). There was no treatment effect for all RE parameters ($P > 0.356$). For RE, Mcal/d and Mcal/kg DMI, QT_3 demonstrated the greatest RE values with 26% more Mcal/kg DMI relative to QT_0. Estimates of RE, Mcal/kg DMI, for QT_0, $QT_{1.5}$, and $QT_{4.5}$ differed only marginally. Retained energy/kg of metabolic body weight did not demonstrate a treatment effect or trend, but QT_3 retained 28% more energy than QT_0. Neither the conversion efficiency of RE (% of MEI) or RE (% of GEI) exhibited treatment differences ($P = 0.375$ and $P = 0.483$). However, QT_3 had the greatest conversion efficiency, demonstrating a 29 and 25% improvement relative to QT_0 for RE %MEI and %GEI, respectively. Treatment $QT_{1.5}$ displayed the lowest conversion efficiency compared to all other treatments. Reduced detection of differences was evident for all RE factors and appeared to be due to animal and period accounting for 21 – 31% of the total variance in the model, with an animal associated variance of FE, UE, and GASE (Mcal/d) ranging from 40 – 93% (Table A-27).

5.4.3. Emissions

The provision of QT reduced the daily production of O_2, CO_2, CH_4, and CO_2e in a linear fashion ($P \leq 0.009$; Table A-28). Total O_2 uptake was lowest for QT_3 ($P = 0.026$), decreasing 5% versus QT_0. On a metabolic body weight basis there was no treatment effect but O_2 consumption decreased linearly ($P = 0.041$). No difference in O_2 consumption was observed when providing 2.5% *Acacia mearnsii* CT while maintaining similar MEI in forage-fed sheep (Carulla et al., 2005). The inclusion of QT above 1.5%

of DM resulted in a 5.5% decrease in total CO_2 ($P < 0.001$) and a 4.5% reduction in CO_2/kg metabolic body weight ($P = 0.001$) compared to QT_0. Daily CH_4 production was only reduced in $QT_{4.5}$ with a 17% reduction in CH_4 observed. Daily CO_2e decreased 6 and 11% for QT_3 and $QT_{4.5}$, respectively, relative to QT_0 ($P = 0.007$). Total CO_2e per OM, aNDF, and ADF intake decreased linearly with increased QT provision ($P \leq 0.007$) with $QT_{4.5}$ having the lowest emissions and $QT_{1.5}$ and QT_3 acting as intermediates for all parameters. However, CO_2e per OM, aNDF, and ADF digested was not different across treatments with no observed trends ($P \geq 0.277$). Retained energy per CO_2e did not demonstrate treatment differences or trends ($P \geq 0.714$). The RE/CO_2e was improved 25% for QT_3 compared to QT_0, with $QT_{1.5}$ being the least efficient. There was no treatment effect or trends for RN/CO_2e ($P \geq 0.962$) with only $QT_{1.5}$ having a discernable reduction relative to all other treatments.

In the present study, QT_3 was the most efficient with increased RE compared to all other treatments. Relative to QT_0, QT_3 had improved RE due to lower HE as FE, UE, and GASE values were comparable across treatments. The reduced HE observed within all QT treatments is likely a result of reduced tissue metabolism associated with ruminal digestibility and route of nutrient absorption, albeit to varying degrees based upon QT level. Within $QT_{4.5}$, lower HE appears to be a consequence of decreased digestibility due to reduced substrate availability depressing microbial activity, product formation, and associated maintenance of visceral tissues. In comparison to QT_0, OM and N digestibilities for $QT_{1.5}$ and QT_3 were reduced to a greater extent than fibrous fractions. This could indirectly indicate rumen escape or protection of precipitable carbohydrates

and proteins, resulting in lesser ruminal degradation and fermentation products. Feeding of CT has resulted in less ruminally degraded NFC and lower total VFA production in previous studies (de Oliveira et al., 2007; Koenig and Beauchemin, 2018). Reducing ruminal digestion would likely reduce energetic costs associated with the digestive and absorptive function of portal-drained viscera as seen when comparing forage vs. concentrate fed animals (Reynolds et al., 1991). Increased O_2 consumption could also be due to time spent eating (Ferrell, 1988) as DMI was greater in all treatments receiving QT extract. However, fermentable OM of diets was isocaloric and isonitrogenous, QT extract was in a powdered form, and all diets were readily consumed. Therefore, HE was likely affected to a larger degree by energetic costs associated with maintenance of portal-drained viscera for digestive and absorptive function rather than the duration of eating or differences in DMI.

Across metabolic parameters, mean estimates for $QT_{1.5}$ and QT_3 were very similar with only slight variation being exhibited. Based upon this observation, provision of QT within the range of 1.5 – 3% may not impart a change in digestive function and efficiency; whereas, the threshold at which QT begins to depress digestion lies between the 3 – 4.5% inclusion rates. The primary mode of action for CT, substrate precipitation, suggests that the threshold at which CT will overwhelm the ruminal ecosystem is based upon the concentration of precipitable carbohydrates and proteins within the diet. When readily precipitated substrate is limited, increased binding of microbial matter will occur since more unbound CT is present in solution. Although scenario dependent, direct inhibition and interaction with ruminal microflora will likely affect digestion to a greater

extent since nutritional requirements for maintenance of microflora populations and energy for microbial processes will be increased during a period of nutrient constraint.

5.5. Conclusion

Quebracho CT inclusion affected metabolic parameters of steers fed a roughage-based diet. The inclusion of QT resulted in less GASE and HE while maintaining similar RE and RN values. A shift in N excretion route was demonstrated, with a larger proportion of N excreted in the feces of animals receiving QT. Altering N excretion route could prove useful in retaining this element within ruminant systems and decrease N_2O and NH_3 emissions associated with urinary N. In total, there was no statistical difference among treatments for RN and RE; however, RE/CO_2e was substantially higher for QT_3 in comparison to all other treatments. Making QT_3 the best prospect for improving system-efficiency while minimizing deleterious effects of animal production. The effects of QT upon nutrient retention and overall efficiency are very dynamic with what appears to be vastly different methods of altering efficiency occurring across a very narrow range of supplementation. However, lack of investigation and understanding of CT-substrate interactions across a vast array of diets hinders our capacity to determine feasible methods of utilization in ruminant production systems.

As emissions from ruminant production are volatile and resultant of metabolic and homeostatic processes, methods emphasizing improved nutrient efficiency defined as nutrient retained/CO_2e should be utilized, rather than attempts to reduce specific gases. Emissions arising from enteric and metabolic processes should not be the only

parameters accounted for when measuring system-efficiency. Since excreta represent the second largest emissions pool that is largely a result of excreta nutrient profile, enumeration, not an estimation, of excreta gas fluxes should be used to determine the most promising management practices, especially when utilizing rumen modulators.

Increased utilization of animal and excreta respirometry should not only quantify how feeding strategies affect animal efficiency and emissions statuses but also explain how different feeding strategies influence nutrient cycling within applied systems. Accordingly, due to the presence of large discrepancies among studies feeding CT to cattle, more investigation of how different CT affect animals at varying growth stages within diverse production settings is required if results are to be utilized in non-research settings. Large among-animal variation and potential carryover effects with CT, as well as other rumen modulators, require that greater animal numbers and cross-over designs be utilized in research.

5.6. References

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6. SUMMARY

6.1. Metabolism and Fecal Gas Flux

The feeding of QT to steers greatly altered metabolic parameters as QT inclusion increased. Provision of QT extract exceeding 3% DM is not recommended under normal conditions as this rate resulted in reduced digestion coefficients and potential gastrointestinal distress. Metabolically, QT_0 and $QT_{1.5}$ did not differ, but $QT_{1.5}$ provides the prospect of improved N_2O emission status through excreta profile alteration. In our study, dietary QT decreased N efficiency and reduced the digestibility of fibrous constituents at 3 and 4.5% QT rates. These reductions would be very deleterious to production when roughage sources comprise the majority of energy within the diet.

Fecal gas fluxes were influenced by QT supplementation. The flux of CO_2 and N_2O, as well as emission factors, were greatly reduced with QT inclusion; however, CH_4 displayed an erratic trend that could potentially be due to high inter-animal variability. Comparison of gross CO_2e per unit DM demonstrated that QT inclusion reduced emissions. When accounting for total daily excretion, the effect of QT upon fecal gas emissions was diminished; however, these estimates are crude since fecal gas fluxes were not representative of the entire sample population and did not include total excreta emissions as urinary N, which represents the greatest proportion of N lost as N_2O-N, was not accounted for.

6.2. Seasonal Fecal Gas Flux

The feeding of QT affected fecal gas emissions depending on environmental conditions. As moisture and temperatures increased from P1 to P2 greater fecal gas fluxes were observed, likely due to environmental conditions conducive to microbial activity. Soil type appears to impart a large influence due to the effect upon soil moisture in the upper profile. The only treatment effects observed were at the College Station study site, as CO_2 and gross CO_2e were reduced at the two highest levels of QT inclusion.

At both locations, substantially greater gas fluxes were observed during P2, with a much larger increase from P1 to P2 at the Stephenville location. However, the College Station site had higher absolute emissions than Stephenville, chiefly driven by temperature differences between locations. These data demonstrate that within warmer environments QT supplementation could potentially reduce more fecal greenhouse-gas emissions than in cooler regions.

6.3. Metabolism and Energy Partitioning

Quebracho CT inclusion affected metabolic parameters of steers fed a roughage-based diet. The inclusion of QT reduced GASE and HE while maintaining similar RE and RN values. The route of N excretion was shifted, with a larger proportion of N being excreted in the feces of animals receiving QT. In total, there was no statistical difference among treatments for RN and RE; however, RE/CO_2e was substantially higher for QT_3

in comparison to all other treatments. Making QT_3 the best prospect for improving system-efficiency while minimizing deleterious effects of animal production.

6.4. Future Research

Emissions from ruminant production are volatile and are the result of metabolic and homeostatic processes. Therefore, methods emphasizing improved nutrient efficiency defined as nutrient retained/CO_2e should be utilized, rather than attempts to reduce specific gases. Emissions arising from enteric and metabolic processes, and excreta should be accounted for when measuring system-efficiency. Since excreta emissions are primarily a result of excreta nutrient profile, enumeration, not an estimation, of gas fluxes is required, especially when utilizing rumen modulators.

Increased utilization of animal and excreta respirometry should not only quantify how feeding strategies affect animal efficiency and emissions statuses but also explain how different feeding strategies influence nutrient cycling within applied systems. Accordingly, due to the presence of large discrepancies among studies feeding CT to cattle, more investigation of how different CT affect animals at varying growth stages within diverse production settings is required if results are to be utilized in non-research settings. Large among-animal variation and potential carryover effects with CT, as well as other rumen modulators, require that greater animal numbers and cross-over designs be utilized in research.

APPENDIX A

TABLES

Table A-1. Ingredient and chemical composition of diets utilized for indigestible content determinations

Items[1]	CON[2]	CT[3]
Ingredient composition, %DM		
Dried distillers grains	39.38	39.38
Cracked corn	39.38	37.13
Bermudagrass hay	13.77	12.15
Molasses	4.48	5.42
Mineral	1.00	0.95
Urea	0.42	0.44
Quebracho extract	--	3.00
Limestone	1.48	1.44
Vitamin E	0.09	0.09
Chemical composition[4]		
DM, %	90.08	90.03
CP, %DM	20.18	19.13
ADF, %DM	10.30	10.65
NDF, %DM	20.00	20.53
NFC, %DM	53.33	53.85
Ash, %DM	6.52	6.58

[1]Items are feed ingredients and chemical composition of diets evaluated by Cumberland Valley Analytical Services (Waynesboro, PA).
[2]CON = control diet
[3]CT = diet containing condensed tannins
[4]DM = dry matter, CP = crude protein, ADF = acid detergent fiber, NDF = neutral detergent fiber, NFC = non-fiber carbohydrates

Table A-2. Mean residues and digestibilities of indigestible dry matter and neutral detergent fiber for three-way interaction of bag type (ANKOM F57 and F58) × sample size (20 and 40 mg/cm^2) × incubation length (288 and 576 h)

		Treatment combinations[2]									
		F57				F58					
		20		40		20		40		SEM[3]	*P*-value
Items	Marker[1]	288	576	288	576	288	576	288	576	(%)	BT x SS x IL[4]
Residues[5], %DM											
Feed-CON	iDM	6.85	5.45	7.21	6.16	7.03	4.84	8.42	6.15	0.22	0.38
	iNDF	4.82	3.55	5.00	3.88	4.65	3.11	5.36	3.78	0.15	0.51
Feed-CT	iDM	7.20	6.62	9.39	7.50	8.55	6.47	9.88	7.21	0.30	0.27
	iNDF	5.23	4.31	6.31	4.92	5.07	3.63	5.91	4.19	0.21	0.59
Feces-CON	iDM	17.74	17.92	19.56	19.27	19.21	19.53	20.74	19.80	1.11	0.60
	iNDF	13.39	12.70	14.57	13.34	14.30	13.28	15.25	13.78	0.76	0.90
Feces-CT	iDM	19.01	20.43	22.58	21.65	22.40	20.94	23.84	21.80	1.1	0.28
	iNDF	14.69	13.49	17.03	14.77	15.93	14.00	16.95	14.86	0.68	0.34
Digestibilities											
DMD-CON	iDM	61.61	70.31	64.65	69.27	64.24	75.96	59.36	69.37	2.25	0.62
	iNDF	63.67	72.08	66.12	71.05	67.11	75.75	63.65	71.78	2.38	0.46
	%Change	2.60	2.31	2.01	2.32	3.41	0.34	4.84	2.95		
DMD-CT	iDM	63.14	67.00	57.76	67.26	63.07	70.26	57.75	66.34	3.45	0.57
	iNDF	63.95	64.06	60.20	63.85	65.12	72.35	61.13	68.50	2.57	0.58
	%Change	-2.91	-3.38	2.00	-3.85	1.61	1.64	2.94	1.71		
NDFD-CON	iDM	43.69	56.42	47.91	54.46	47.09	64.55	40.94	54.91	3.83	0.70
	iNDF	47.96	60.16	51.52	58.56	52.89	65.65	48.43	59.83	3.09	0.51
	%Change	3.80	3.28	3.14	3.63	5.34	0.63	7.02	4.46		
NDFD-CT	iDM	52.93	56.30	44.27	58.02	52.01	61.11	45.16	55.46	5.79	0.33
	iNDF	49.37	51.96	47.18	53.27	53.91	63.35	48.90	57.79	4.98	0.60
	%Change	-3.58	-4.36	2.88	-4.77	1.86	2.21	3.71	2.30		

[1]iDM = indigestible dry matter, iNDF = indigestible neutral detergent fiber, %Change = percent change in digestibility from iDM to iNDF

[2] Bag type: 57 = 25 µm bag and F58 = 10 µm bag (ANKOM technology, Macedon, NY), Sample size: 20 and 40 mg/cm^2, Incubation length: 288 and 576 h

[3]SEM = standard error of the mean

[4]Bag type x Sample size x Incubation length

[5]Residue values are percentages: CON = control diet, CT = condensed tannin diet; Digestibility estimates are percentages: DMD = DM digestibility, NDFD = NDF digestibility

Table A-3. Mean residues and digestibilities of iDM and iNDF for the interaction of bag type (ANKOM F57 and F58) and sample size (20 and 40 mg/cm^2)

		Treatment combinations[2]					
		F57		F58		SEM[3]	
Items	Marker[1]	20	40	20	40	(%)	*P*-value
Residues[4], %DM							
Feed-CON	iDM	6.14^{C}	6.68^{B}	5.93^{C}	7.28^{A}	0.18	<0.01
	iNDF	4.18^{B}	4.44^{A}	3.88^{C}	4.57^{A}	0.13	<0.01
Feed-CT	iDM	6.91	8.44	7.51	8.54	0.24	0.13
	iNDF	4.77	5.62	4.35	5.05	0.19	0.40
Feces-CON	iDM	17.83	19.41	19.37	20.27	1.04	0.36
	iNDF	13.05	13.95	13.79	14.52	0.72	0.66
Feces-CT	iDM	19.72	22.12	21.67	22.82	1.02	0.13
	iNDF	14.09	15.90	14.97	15.90	0.63	0.07
Digestibilities[5]							
DMD-CON	iDM	65.96^{B}	66.96^{AB}	70.10^{A}	64.37^{B}	1.91	<0.01
	iNDF	67.88^{B}	68.59^{B}	71.43^{A}	67.72^{B}	2.17	0.03
	%Change[6]	2.8	2.4	1.9	4.9		
DMD-CT	iDM	65.07	62.51	66.66	62.05	2.92	0.58
	iNDF	64.01	62.03	68.74	64.81	1.86	0.52
	%Change	-1.7	-0.8	3.0	4.3		
NDFD-CON	iDM	50.05^{B}	51.18^{AB}	55.82^{A}	47.92^{B}	3.42	0.01
	iNDF	54.06^{B}	55.04^{B}	59.27^{A}	54.13^{B}	2.74	0.03
	%Change	7.4	7.0	5.8	11.%		
NDFD-CT	iDM	54.62	51.15	56.56	50.31	5.30	0.55
	iNDF	50.67	50.23	58.63	53.35	4.60	0.21
	%Change	-7.8	-1.8	3.5	5.7		

$^{A-D}$ Means followed by different superscripts within rows differ according to least significant difference ($P \leq 0.05$).
[1]iDM = indigestible dry matter, iNDF = indigestible neutral detergent fiber
[2] Bag type: 57 = 25 μm bag and F58 = 10 μm bag (ANKOM technology, Macedon, NY), Sample size: 20 and 40 mg/cm^2
[3]SEM = standard error of the mean
[4]All residue values are percentages: CON = control diet, CT = condensed tannin diet
[5]All digestibility estimates are percentages: DMD = dry matter digestibility, NDFD = neutral detergent fiber digestibility
[6]Percent change in digestibility estimate from iDM to iNDF

Table A-4. Mean residues and digestibilities of iDM and iNDF for the interaction of bag type (ANKOM F57 and F58) and incubation length (288 and 576 h)

		Treatment combinations[2]					
		F57		F58		SEM[3]	
Items	Marker[1]	288	576	288	576	(%)	*P*-value
Residues[4], %DM							
Feed-CON	iDM	7.02^{B}	5.80^{C}	7.72^{A}	5.49^{C}	0.18	<0.01
	iNDF	4.91^{A}	3.71^{B}	5.01^{A}	3.45^{C}	0.13	<0.01
Feed-CT	iDM	8.29^{B}	7.06^{C}	9.22^{A}	6.84^{C}	0.24	<0.01
	iNDF	5.77^{A}	4.62^{C}	5.49^{B}	3.91^{D}	0.19	0.01
Feces-CON	iDM	18.65	18.59	19.97	19.66	1.04	0.73
	iNDF	13.98	13.02	14.77	13.53	0.72	0.49
Feces-CT	iDM	20.80^{B}	21.04^{B}	23.12^{A}	21.37^{B}	1.02	0.01
	iNDF	15.86	14.13	16.44	14.43	0.63	0.55
Digestibilities[5]							
DMD-CON	iDM	63.13	69.79	61.80	72.67	1.91	0.08
	iNDF	64.89	71.57	65.38	73.77	2.17	0.39
	%Change[6]	2.7	2.5	5.5	1.5		
DMD-CT	iDM	60.45	67.13	60.41	68.30	2.92	0.74
	iNDF	62.08	63.96	63.13	70.42	1.86	0.08
	%Change	2.6	-5.0	4.3	3.0		
NDFD-CON	iDM	45.80	55.44	44.02	59.73	3.42	0.08
	iNDF	49.74	59.36	50.66	62.74	2.74	0.39
	%Change	7.9	6.6	13.1	4.8		
NDFD-CT	iDM	48.60	57.16	48.59	58.28	5.30	0.8
	iNDF	48.28	52.62	51.41	60.57	4.60	0.21
	%Change	-0.7	-8.6	5.5	3.8		

[A-D] Means followed by different superscripts within rows differ according to least significant difference ($P \leq 0.05$).
[1]iDM = indigestible dry matter, iNDF = indigestible neutral detergent fiber
[2] Bag type: 57 = 25 μm bag and F58 = 10 μm bag (ANKOM technology, Macedon, NY), Incubation length: 288 and 576 h
[3]SEM = standard error of the mean
[4]All residue values are percentages: CON = control diet, CT = condensed tannin diet
[5]All digestibility estimates are percentages: DMD = dry matter digestibility, NDFD = neutral detergent fiber digestibility
[6]Percent change in digestibility from iDM to iNDF

Table A-5. Mean residues and digestibilities of iDM and iNDF for the interaction of sample size (20 and 40 mg/cm^2) and incubation length (288 and 576 h)

		Treatment combinations[2]					
		20		40		SEM[3]	
Items	Marker[1]	288 h	576 h	288 h	576 h	(%)	*P*-value
Residues[4], %DM							
Feed-CON	iDM	6.93	5.14	7.81	6.15	0.18	0.58
	iNDF	4.74	3.33	5.18	3.83	0.13	0.71
Feed-CT	iDM	7.87^{B}	6.54^{D}	9.63^{A}	7.35^{C}	0.24	<0.01
	iNDF	5.15^{B}	3.97^{D}	6.11^{A}	4.56^{C}	0.19	0.03
Feces-CON	iDM	18.47	18.72	20.15	19.53	1.04	0.24
	iNDF	13.85	12.99	14.91	13.56	0.72	0.24
Feces-CT	iDM	20.70	20.68	23.21	21.72	1.02	0.07
	iNDF	15.31	13.74	16.99	14.81	0.63	0.19
Digestibilities[5]							
DMD-CON	iDM	62.93	73.14	62.01	69.32	1.91	0.23
	iNDF	65.39	73.92	64.89	71.42	2.17	0.31
	%Change[6]	3.8	1.1	4.4	2.9		
DMD-CT	iDM	63.10	68.63	57.76	66.80	2.92	0.34
	iNDF	64.54	68.21	60.67	66.18	1.86	0.54
	%Change	2.2	-0.6	4.8	-0.9		
NDFD-CON	iDM	45.39	60.48	44.42	54.68	3.42	0.17
	iNDF	50.43	62.90	49.97	59.20	2.74	0.26
	%Change	10.0	3.8	11.1	7.6		
NDFD-CT	iDM	52.47	58.71	44.72	56.74	5.30	0.22
	iNDF	51.64	57.66	48.04	55.53	4.60	0.70
	%Change	-1.6	-1.8	6.9	-2.2		

[A-D] Means followed by different superscripts within rows differ according to least significant difference ($P \leq 0.05$).
[1]iDM = indigestible dry matter, iNDF = indigestible neutral detergent fiber
[2]Sample size: 20 and 40 mg/cm^2, Incubation length: 288 and 576 h
[3]SEM = standard error of the mean
[4]All residue values are percentages: CON = control diet, CT = condensed tannin diet
[5]All digestibility estimates are percentages: DMD = dry matter digestibility, NDFD = neutral detergent fiber digestibility
[6]Percent change in digestibility from iDM to iNDF

Table A-6. Mean residues and digestibilities of iDM and iNDF for main effects of bag type (ANKOM F57 and F58) × sample size (20 and 40 mg/cm^2) × incubation length (288 and 576 h)

		Main effects[2]									
		Bag		Sample size		Incubation length			*P*-value		
Items	Marker[1]	F57	F58	20[2]	40	288-h	576-h	SEM[3] (%)	BT	SS	IL[4]
Residues[5], %DM											
Feed-CON	iDM	6.41	6.61	6.04	6.98	7.37	5.65	0.16	0.11	<0.01	<0.01
	iNDF	4.31	4.23	4.03	4.50	4.96	3.58	0.12	0.21	<0.01	<0.01
Feed-CT	iDM	7.67	8.03	7.21	8.49	8.75	6.95	0.21	0.03	<0.01	<0.01
	iNDF	5.19	4.70	4.56	5.34	5.63	4.26	0.18	<0.01	<0.01	<0.01
Feces-CON	iDM	18.62	19.82	18.60	19.84	19.31	19.13	1.24	<0.01	<0.01	0.62
	iNDF	13.50	14.15	13.42	14.24	14.38	13.28	0.71	<0.01	<0.01	<0.01
Feces-CT	iDM	20.92	22.24	20.69	22.47	21.96	21.20	0.97	<0.01	<0.01	0.07
	iNDF	14.99	15.44	14.53	15.90	16.15	14.28	0.61	0.06	<0.01	<0.01
Digestibilities											
DMD-CON	iDM	66.46	67.23	68.03	65.67	62.47	71.23	1.71	0.52	0.055	<0.01
	iNDF	68.23	69.57	69.65	68.15	65.14	72.67	2.05	0.18	0.13	<0.01
	%Change[6]	2.31	2.88	2.17	3.03	3.21	1.98				
DMD-CT	iDM	63.79	64.36	65.87	62.28	60.43	67.72	2.61	0.76	0.06	<0.01
	iNDF	63.02	66.78	66.37	63.42	62.60	67.19	1.45	0.01	0.06	<0.01
	%Change	-2.03	1.97	-0.75	0.70	0.91	-0.96				
NDFD-CON	iDM	50.62	51.87	52.94	49.55	44.91	57.58	3.19	0.47	0.05	<0.01
	iNDF	54.55	56.70	56.66	54.59	50.20	61.05	2.55	0.14	0.15	<0.01
	%Change	3.46	4.36	3.26	4.57	4.83	3.00				
NDFD-CT	iDM	52.88	53.43	55.59	50.73	48.59	57.72	5.04	0.81	0.04	<0.01
	iNDF	50.45	55.99	54.65	51.79	49.84	56.59	4.39	<0.01	0.14	<0.01
	%Change	-2.46	2.52	-0.96	1.03	1.22	-1.15				

[1]iDM = indigestible dry matter, iNDF = indigestible neutral detergent fiber, %Change = percent change in digestibility from iDM to iNDF

[2] Bag type: 57 = 25 μm bag and F58 = 10 μm bag (ANKOM technology, Macedon, NY), Sample size: 20 and 40 mg/cm^2, Incubation length: 288 and 576 h

[3]SEM = standard error of the mean

[4]Bag type x Sample size x Incubation length

[5]Residue values are percentages: CON = control diet, CT = condensed tannin diet; Digestibility estimates percentages: DMD = DM digestibility, NDFD = NDF digestibility

Table A-7. Variance partitioning of random effects

Items[1]	Marker[2]	Incubation animal	Animal	Period	Residual
Feed-CON	iDM	1.1%	N/A	N/A	98.9%
	iNDF	4.1%	N/A	N/A	95.9%
Feed-CT	iDM	0.7%	N/A	N/A	99.3%
	iNDF	3.3%	N/A	N/A	96.7%
Feces-CON	iDM	2.8%	N/A	N/A	97.2%
	iNDF	0.6%	N/A	N/A	99.4%
Feces-CT	iDM	2.1%	N/A	N/A	97.9%
	iNDF	0.0%	N/A	N/A	100.0%
DMD-CON	iDM	N/A	43.8%	0.0%	56.2%
	iNDF	N/A	6.5%	50.1%	43.3%
DMD-CT	iDM	N/A	19.8%	15.6%	64.6%
	iNDF	N/A	7.4%	0.8%	91.8%
NDFD-CON	iDM	N/A	5.7%	43.8%	50.5%
	iNDF	N/A	45.9%	2.1%	52.0%
NDFD-CT	iDM	N/A	9.9%	49.2%	40.9%
	iNDF	N/A	15.1%	47.4%	37.5%

[1]CON = control diet, CT = condensed tannin diet, DMD = dry matter digestibility, NDFD = neutral detergent fiber digestibility
[2]iDM = indigestible dry matter, iNDF = indigestible neutral detergent fiber
[3]N/A = not applicable

Table A-8. Ingredient and chemical composition of the high-roughage total-mixed ration utilized for metabolism and manure gas flux

	Basal diet
Items[1]	%
Ingredient composition, % DM	
Cottonseed hulls	37.00
Cracked corn	33.00
Alfalfa pellets	11.50
Bermudagrass hay	8.00
Molasses	7.00
Mineral	2.50
Urea	1.00
Chemical composition[2]	
DM, %	86.60
CP, % DM	12.40
Soluble protein, % CP	39.70
aNDF, % DM	48.10
ADF, % DM	33.40
Lignin, % DM	9.81
Crude fat, % DM	2.29
Sugar, % DM	6.10
Starch, % DM	21.50
NFC, % DM	34.20
Ash, % DM	6.52
Calcium	0.72
Phosphorus	0.40
TDN, %	58.40
NE_m, Mcal/kg	1.21
NE_g, Mcal/kg	0.64
GE, Mcal/kg[3]	3.99

[1]Items are feed ingredients and chemical composition of diets were evaluated by Cumberland Valley Analytical Services (Waynesboro, PA).
[2]DM = dry matter; CP = crude protein; aNDF = neutral detergent fiber with amylase and sodium sulfate; ADF = acid detergent fiber; NFC = non-fiber carbohydrates; NEm = net energy for maintenance; NEg = net energy for gain
[3]GE measured by bomb calorimeter

Table A-9. Effect of quebracho tannin treatment on consumption and excretion profiles

	Quebracho extract, % of DM						Contrast[1] *P*- value		
Items[2]	0[3]	1.5	3	4.5	SEM[4]	*P*-value	L	Q	C
SBW, kg	433.38	434.38	439.25	434.38	8.14	0.342	0.477	0.242	0.225
Water intake, kg/d	11.14	10.90	11.37	12.08	1.56	0.921	0.570	0.719	0.937
Water intake, % SBW	2.60	2.52	2.58	2.78	0.36	0.936	0.662	0.652	0.996
Water intake: DMI	1.57	1.52	1.56	1.71	0.22	0.901	0.599	0.605	0.981
DMI, kg.d	7.15^{AB}	7.18^{AB}	7.26^{A}	7.08^{B}	0.12	0.018	0.358	0.009	0.075
DMI, %SBW	1.65^{A}	1.65^{A}	1.65^{A}	1.63^{B}	0.70	0.003	0.011	0.004	0.577
Fecal DM, kg/d	2.47^{C}	2.71^{BC}	3.13^{AB}	3.31^{A}	0.13	<0.001	<0.001	0.816	0.395
Fecal DM, %SBW	0.57^{C}	0.62^{BC}	0.71^{AB}	0.76^{A}	0.003	<0.001	<0.001	0.980	0.524
Fecal DM, %	24.28	24.04	23.22	24.18	0.85	0.671	0.711	0.378	0.440
Urine, kg/d	10.01	8.42	6.74	8.74	1.80	0.104	0.176	0.055	0.346
Urine, %SBW	2.31	1.95	1.53	2.00	0.40	0.079	0.141	0.048	0.302

[A-C]Least Squares means in a row with different superscripts differ at $P \leq 0.05$
[1]Contrasts: L = linear, Q = quadratic, C = cubic
[2]SBW = shrunk body weight; DMI = dry matter intake
[3]Quebracho extract contained 28.9% protein precipitable phenolics
[4]SEM = standard error of the mean

Table A-10. Effect of quebracho tannin treatment on digestibility and nitrogen metabolism

	Quebracho extract, % of DM						Contrast[1] *P*- value		
Items[2]	0[3]	1.5	3	4.5	SEM[4]	*P*-value	L	Q	C
DMD, %	65.28^{A}	62.24^{AB}	56.80^{BC}	52.99^{C}	1.92	<0.001	<0.001	0.803	0.564
NDFD, %	57.07^{A}	54.99^{AB}	48.96^{BC}	44.70^{C}	3.23	<0.001	<0.001	0.579	0.514
ADFD, %	45.46^{A}	42.33^{AB}	34.71^{BC}	28.81^{C}	4.75	<0.001	<0.001	0.596	0.593
Apparent N digestibility, %	54.51^{A}	47.55^{AB}	40.46^{BC}	36.97^{C}	3.60	<0.001	<0.001	0.377	0.668
Fecal N, g/d	55.98^{C}	65.26^{BC}	74.57^{AB}	77.21^{A}	3.49	<0.001	<0.001	0.187	0.543
Fecal N concentration, %	2.26	2.41	2.37	2.34	0.06	0.052	0.210	0.022	0.244
Urine N, g/d	35.10	33.37	23.16	28.23	4.84	0.150	0.087	0.384	0.180
Urine N concentration, %	0.42	0.42	0.36	0.41	0.07	0.714	0.616	0.576	0.384
Total N excreted, g/d	91.08	98.64	97.74	105.45	6.37	0.231	0.058	0.987	0.430
Retained N, g/d	33.02	26.21	28.28	17.63	8.18	0.192	0.057	0.697	0.334
Retained N, %	26.12	20.35	22.06	14.03	6.20	0.217	0.065	0.777	0.341
Fecal N, % N excreted	63.01^{B}	67.16^{AB}	76.62^{A}	73.5^{A}	3.46	0.005	0.001	0.164	0.128
Fecal N:Urinary N	1.93^{B}	2.30^{B}	3.72^{A}	3.07^{AB}	0.55	0.007	0.005	0.156	0.056

[A-C]Least Squares means in a row with different superscripts differ at $P \leq 0.05$

[1]Contrasts: L = linear, Q = quadratic, C = cubic

[2]DMD = dry matter digestibility; NDFD = neutral detergent fiber digestibility; ADFD = acid detergent fiber digestibility; N = nitrogen

[3]Quebracho extract contained 28.9% protein precipitable phenolics

[4]SEM = standard error of the mean

Table A-11. Effect of quebracho tannin treatment on energy metabolism

	Quebracho extract, % of DM						Contrast[1] *P*- value		
Items[2]	0[3]	1.5	3	4.5	SEM[4]	*P*-value	L	Q	C
GEI, Mcal/d	28.58^{C}	29.23^{B}	30.06^{A}	29.86^{B}	0.21	<0.001	<0.001	0.010	0.083
FE, Mcal/d	10.24^{C}	11.22^{BC}	13.03^{AB}	13.77^{A}	0.67	<0.001	<0.001	0.800	0.385
FE, Mcal/kg	4.13	4.13	4.15	4.15	0.04	0.852	0.414	0.881	0.806
UE, Mcal/d	0.92	0.83	0.70	0.80	0.10	0.271	0.174	0.214	0.454
UE, Mcal/kg	0.10	0.10	0.10	0.10	0.01	0.950	0.767	0.733	0.717
Total E excreted, Mcal/d	11.16^{C}	12.05^{BC}	13.73^{AB}	14.58^{A}	0.71	<0.001	<0.001	0.964	0.479
DE, Mcal/d	18.33^{A}	18.00^{A}	17.02^{AB}	16.08^{B}	0.64	0.011	0.001	0.516	0.744
DE, Mcal/kg DM	2.55^{A}	2.50^{AB}	2.34^{AB}	2.25^{B}	0.09	0.016	0.002	0.783	0.565
DE, %GE	64.09^{A}	61.60^{AB}	56.62^{BC}	53.67^{C}	2.10	<0.001	<.0001	0.881	0.527
FE, % E excreted	91.75^{B}	93.11^{AB}	94.91^{A}	94.51^{A}	0.77	0.004	<0.001	0.156	0.334
FE:UE	12.72^{B}	14.31^{AB}	20.29^{A}	18.41^{AB}	2.41	0.015	0.005	0.320	0.121

[A-C]Least Squares means in a row with different superscripts differ at $P \leq 0.05$

[1]Contrasts: L = linear, Q = quadratic, C = cubic

[2]GEI = gross energy intake; FE = fecal energy; UE = urinary energy; DE = digestible energy

[3]Quebracho extract contained 28.9% protein precipitable phenolics

[4]SEM = standard error of the mean

Table A-12. Effect of quebracho tannin treatment on rumen and blood parameters

	Quebracho extract, % of DM						Contrast[1] *P*- value		
Items[2]	0[3]	1.5	3	4.5	SEM[4]	*P*-value	L	Q	C
pH	6.67	6.84	6.82	6.72	0.07	0.287	0.727	0.062	0.774
Protozoa, log_{10}/mL	5.84	5.56	5.76	5.74	0.88	0.076	0.779	0.087	0.039
NH_3-N, mg/L	68.22	58.51	55.19	45.13	8.95	0.0888	0.014	0.976	0.629
Total VFA, mmol/L	35.80	32.72	36.59	34.78	2.55	0.633	0.933	0.774	0.210
Acetate, %	57.62	59.38	56.65	56.28	1.43	0.418	0.292	0.453	0.283
Propionate, %	18.68	17.72	18.18	19.15	0.88	0.682	0.634	0.280	0.815
Isobutyrate, %	1.82	1.89	1.77	1.60	0.12	0.237	0.098	0.238	0.731
Butyrate, %	15.62	15.83	16.10	17.17	1.04	0.366	0.107	0.519	0.801
Isovalerate, %	4.55	3.48	5.48	4.26	0.55	0.110	0.648	0.892	0.018
Valerate, %	1.72	1.70	1.82	1.54	0.10	0.280	0.369	0.200	0.232
Acetate:Propionate	3.17	3.43	3.14	3.03	0.21	0.593	0.467	0.389	0.436
Glucose, mg/dl	63.50	63.38	62.75	61.38	3.76	0.933	0.551	0.811	0.983
Albumin, g/dl	3.03	3.01	2.98	2.98	0.07	0.889	0.466	0.91	0.807
BUN, mg/dl	6.25^{A}	5.50^{AB}	4.87^{B}	5.00^{AB}	0.62	0.031	0.007	0.200	0.677
Total protein, g/dl	6.30	6.54	6.49	6.49	0.15	0.396	0.277	0.261	0.470
Creatinine, mg/dl	1.34	1.34	1.39	1.38	0.04	0.575	0.260	0.843	0.431

[A-C]Least Squares means in a row with different superscripts differ at $P \leq 0.05$

[1]Contrasts: L = linear, Q = quadratic, C = cubic

[2]NH_3-N = ammonia nitrogen; VFA = volatile fatty acids; BUN = blood urea nitrogen

[3]Quebracho extract contained 28.9% protein precipitable phenolics

[4]SEM = standard error of the mean

Table A-13. Effect of quebracho tannin treatment on cumulative fecal gas flux

	Quebracho extract, % of DM						Contrast[1] *P*- value		
Items[2]	0[3]	1.5	3	4.5	SEM[4]	*P*-value	L	Q	C
CO_2, mg/g DM	154.18^{A}	142.33^{A}	108.00^{B}	101.79^{B}	10.77	0.063	0.017	0.806	0.353
CH_4, mg/g DM	0.21^{B}	0.37^{A}	0.14^{C}	0.29^{A}	0.06	0.006	0.746	0.819	0.032
N_2O, µg/g DM	2.73^{A}	2.23^{A}	0.80^{B}	0.43^{B}	0.30	<0.001	<0.001	0.840	0.171
Total CO_2e, mg/g DM	6.46^{A}	10.98^{A}	4.24^{B}	8.32^{A}	1.99	0.008	0.697	0.849	0.032
Gross CO_2e, mg/g DM	160.65^{A}	153.31^{A}	112.23^{B}	110.10^{B}	11.43	0.068	0.020	0.831	0.228
Emission Factor, µg/g									
total N	72.24^{A}	54.34^{B}	20.01^{C}	10.62^{C}	6.75	<0.001	<0.001	0.543	0.195
soluble N	367.91^{A}	330.01^{A}	110.21^{B}	77.46^{B}	45.17	0.021	0.005	0.957	0.141

[A-C]Least Squares means in a row with different superscripts differ at P ≤ 0.10

[1]Contrasts: L = linear, Q = quadratic, C = cubic

[2]CO_2 = carbon dioxide; CH_4 = methane; N_2O = nitrous oxide; Total CO_2e = CO_2 equivalent emissions (CH_4 + N_2O); Gross CO_2e = CO_2 equivalent emissions (CO_2 + CH_4 + N_2O); Emission factor = proportion of fecal nitrogen emitted as N2O-N

[3]Quebracho extract contained 28.9% protein precipitable phenolics

[4]SEM = standard error of the mean

Table A-14. Correlation coefficients for fecal nutrients and gas fluxes

Items[1]	N	Soluble N	NDF	ADF	NFC	CO_2	CH_4	N_2O	Total CO_2e	Gross CO_2e
N	1.000[A]									
Soluble N	0.004	1.000								
NDF	0.862**	0.076	1.000							
ADF	0.895**	0.074	0.983**	1.000						
NFC	0.486*	0.765**	0.34	0.334	1.000					
CO_2	-0.283	0.766**	-0.206	-0.247	0.508*	1.000				
CH_4	-0.171	-0.146	-0.517*	-0.474*	0.184	0.005	1.000			
N_2O	-0.358	0.672**	-0.367	-0.418	0.422	0.741**	-0.019	1.000		
Total CO_2e	-0.21	-0.071	-0.555*	-0.519*	0.231	0.087	0.993**	0.092	1.000	
Gross CO_2e	-0.298	0.75**	-0.253	-0.291	0.523*	0.995**	0.095	0.74**	0.177	1.000

[A]Coefficient of correlation
* = $P \leq 0.05$; ** = $P \leq 0.01$
[1] N = nitrogen; NDF = neutral detergent fiber; ADF = acid detergent fiber; NFC = non-fibrous carbohydrates; CO_2 = carbon dioxide; CH_4 = methane; N_2O = nitrous oxide; Total CO_2e = CO_2 equivalent emissions (CH_4 + N_2O); Gross CO_2e = CO_2 equivalent emissions (CO_2 + CH_4 + N_2O)

Table A-15. Effect of quebracho tannin treatment on estimated cumulative manure gas flux from daily fecal excretion[1]

	Quebracho extract, % of DM						Contrast[2] *P*- value		
Items[3]	0[4]	1.5	3	4.5	SEM[5]	*P*-value	L	Q	C
CO_2, g	382.22^{A}	386.19^{A}	339.00^{A}	337.51^{A}	18.41	0.020	0.005	0.843	0.110
CH_4, g	0.50^{B}	1.00^{A}	0.45^{B}	0.97^{A}	0.04	<0.001	<0.001	0.737	<0.001
N_2O, mg	6.75^{A}	6.03^{B}	2.50^{C}	1.40^{D}	0.22	<0.001	<0.001	0.246	<0.001
Total CO_2e, g	16.02^{B}	29.79^{A}	13.28^{B}	27.57^{A}	1.21	<0.001	<0.001	0.764	<0.001
Gross CO_2e, g	398.25^{AB}	415.99^{A}	352.00^{B}	365.06^{AB}	19.55	0.015	0.016	0.867	0.019
N2O-N, mg	4.01^{A}	3.51^{B}	1.48^{C}	0.83^{D}	0.13	<0.001	<0.001	0.440	<0.001

[A-D]Least Squares means in a row with different superscripts differ at $P \leq 0.05$ in accordance with the Tukey-Kramer test

[1]Estimates calculated from daily fecal data from metabolism trial and cumulative manure gas flux per unit DM

[2]Contrasts: L = linear, Q = quadratic, C = cubic

[3]CO2 = carbon dioxide; CH_4 = methane; N_2O = nitrous oxide; Total CO_2e = CO_2 equivalent emissions (CH_4 + N_2O); Gross CO_2e = CO_2 equivalent emissions (CO_2 + CH_4 + N_2O); N_2O-N = total nitrogen emitted as N_2O

[4]Quebracho extract contained 28.9% protein precipitable phenolics

[5]SEM = standard error of the mean

Table A-16. Ingredient and chemical composition of the high-roughage total-mixed ration utilized for fecal gas flux

	Basal Diet
Items[1]	%
Ingredient composition, % DM	
Cottonseed hulls	37.00
Cracked corn	33.00
Alfalfa pellets	11.50
Bermudagrass hay	8.00
Molasses	7.00
Mineral	2.50
Urea	1.00
Chemical composition[2]	
DM, %	87.20
CP, %DM	12.90
Soluble protein, %CP	45.05
aNDF, %DM	48.70
ADF, %DM	35.85
Lignin, %DM	10.27
Crude fat, %DM	3.21
Sugar, %DM	3.30
Starch, %DM	20.30
NFC, %DM	32.00
Ash, %DM	5.86
Calcium	0.75
Phosphorus	0.43
TDN, %	60.95
NE_m, Mcal/kg	1.32
NE_g, Mcal/kg	0.76
GE, Mcal/kg[3]	3.85

[1]Items are feed ingredients and chemical composition of diets evaluated by Cumberland Valley Analytical Services (Waynesboro, PA).
[2]DM = dry matter; CP = crude protein; aNDF = neutral detergent fiber with amylase and sodium sulfate; ADF = acid detergent fiber; NFC = non-fiber carbohydrates; NE_m = net energy for maintenance; NEg = net energy for gain
[3]GE measured by bomb calorimeter

Table A-17. Chemical composition of feces utilized for fecal gas flux enumeration

	Period 1[1]				Period 2			
	Dietary Quebracho extract, % of DM				Dietary Quebracho extract, % of DM			
Chemical composition[2]	0[3]	1.5	3	4.5	0	1.5	3	4.5
DM, %	23.25	23.39	25.02	22.31	22.93	24.24	23.51	23.29
Crude protein, %	15.40	16.30	16.63	15.97	15.00	15.53	15.90	14.77
Soluble protein, %CP	13.00	13.40	17.73	13.63	12.23	12.13	10.37	10.67
NDF, %DM	58.40	56.40	54.50	55.30	59.43	58.90	61.97	59.43
ADF, %DM	51.57	49.97	48.40	49.73	54.43	53.03	55.17	52.17
NFC, %DM	15.70	18.00	20.30	19.43	14.90	14.77	11.93	16.07
Ash, %DM	10.48	9.25	8.60	9.27	10.67	10.77	10.22	9.70
Extractable CT, %DM	0.45	1.17	1.11	1.48	0.22	0.31	0.57	0.91
Protein bound CT, %DM	6.57	7.16	6.96	8.13	5.51	5.16	5.58	6.44
Fiber bound CT, %DM	3.16	2.71	3.31	2.83	2.45	3.21	2.66	2.69
Total CT, %DM	10.19	11.04	11.38	12.45	8.18	8.68	8.80	10.05

[1]Period 1 corresponds with winter; Period 2 corresponds with spring
[2]Chemical composition of diets evaluated by Cumberland Valley Analytical Services (Waynesboro, PA). DM = dry matter; CP = crude protein; aNDF = neutral detergent fiber with amylase and sodium sulfate; ADF = acid detergent fiber; NFC = non-fiber carbohydrates; CT = condensed tannins
[3]Quebracho extract contained 28.9% protein precipitable phenolics

Table A-18. Correlation coefficients for environmental parameters and daily fecal gas fluxes

Items[1]	Location	Period	CO_2	CH_4	N_2O	Gross CO_2e	VWC	Soil temperature
Location	1.000[A]							
Period	-0.033	1.000						
CO_2	-0.273*	0.395*	1.000					
CH_4	0.013	0.161*	0.409*	1.000				
N_2O	-0.027	0.044	0.195*	0.021	1.000			
Gross CO_2e	-0.260*	0.393*	0.996*	0.483*	0.196*	1.000		
VWC	0.337*	0.213*	0.340*	0.237*	0.126*	0.348*	1.000	
Soil temperature	-0.132*	0.842*	0.348*	-0.027	0.0447	0.331*	0.049	1.000

[A]Coefficient of correlation
* = $P \leq 0.05$
[1] Location = College Station (1) and Stephenville (2); Period 1 spanned 1 January 2018 – 16 February 2018, Period 2 spanned 9 April 2018 – 18 May 2018; CO_2 = carbon dioxide; CH_4 = methane; N_2O = nitrous oxide; Gross CO_2e = CO_2 equivalent emissions (CO_2 + CH_4 + N_2O); VWC = soil volumetric water content

Table A-19. Effect of quebracho tannin on cumulative fecal gas flux at the College Station location

	Quebracho extract, % of DM					Period[1]			*P*-value[2]			Contrast[3] *P*-value		
Items[4]	0[5]	1.5	3	4.5	SEM[6]	1	2	SEM	T	P	T × P	L	Q	C
CO_2	138.90^{A}	136.61^{A}	125.02^{AB}	110.62^{B}	5.92	100.97	154.61	4.19	0.014	<0.001	0.120	0.002	0.323	0.809
CH_4	0.07	0.04	0.05	0.05	0.01	0.04	0.07	0.01	0.678	0.025	0.631	0.619	0.381	0.501
N_2O	0.78	0.67	0.62	0.49	0.22	0.44	0.84	0.15	0.836	0.079	0.395	0.3672	0.954	0.896
Total CO_2e	2.20	1.45	1.76	1.72	0.40	1.22	2.33	0.28	0.626	0.014	0.704	0.539	0.395	0.440
Gross CO_2e	141.10^{A}	138.06^{A}	126.78^{AB}	112.34^{B}	6.08	102.20	156.95	4.30	0.016	<0.001	0.148	0.002	0.363	0.854
Emission factor														
Total N	3.31	2.66	2.46	2.06	0.887	1.75	3.50	0.62	0.791	0.054	0.410	0.325	0.888	0.872
Soluble N	27.95	20.43	23.22	18.58	8.52	12.92	32.17	6.03	0.876	0.029	0.406	0.511	0.867	0.645

[A-C]Least Squares means in a row with different superscripts differ at $P \leq 0.05$

[1]Period 1 spanned 1 January 2018 – 16 February 2018; Period 2 spanned 9 April 2018 – 18 May 2018

[2]T = dietary treatment effect; P = period effect; T × P = dietary treatment and period interaction

[3]Contrasts: L = linear, Q = quadratic, C = cubic

[4]CO_2 = carbon dioxide, mg/g DM; CH_4 = methane, mg/g DM; N_2O = nitrous oxide, µg/g DM; Total CO_2e = CO_2 equivalent emissions (CH_4 + N_2O) , mg/g DM; Gross CO_2e = CO_2 equivalent emissions (CO_2 + CH_4 + N_2O) , mg/g DM; Emission factor = proportion of fecal nitrogen emitted as N_2O-N, µg/g

[5]Quebracho extract contained 28.9% protein precipitable phenolics

[6]SEM = standard error of the mean

Table A-20. Effect of quebracho tannin on cumulative fecal gas flux at the Stephenville location

	Quebracho extract, % of DM					Period[1]			*P*-value[2]			Contrast[3] *P*-value		
Items[4]	0[5]	1.5	3	4.5	SEM[6]	1	2	SEM	T	P	T × P	L	Q	C
CO_2	68.08	66.36	58.77	67.25	9.00	20.00	110.23	6.37	0.876	<0.001	0.883	0.805	0.579	0.594
CH_4	0.06	0.04	0.06	0.05	0.01	0.02	0.08	0.01	0.743	<0.001	0.744	0.877	0.604	0.346
N_2O	0.33	0.30	0.35	0.53	0.12	0.17	0.59	0.12	0.792	0.029	0.616	0.415	0.555	0.936
Total CO_2e	1.86	1.31	1.80	1.67	0.39	0.73	2.59	0.27	0.748	<0.001	0.755	0.975	0.595	0.349
Gross CO_2e	69.94	67.67	60.57	69.00	9.03	20.78	112.82	6.38	0.880	<0.001	0.883	0.809	0.562	0.622
Emission factor														
Total N	1.45	1.27	1.35	2.85	0.74	0.98	2.47	0.52	0.771	0.026	0.592	0.462	0.465	0.863
Soluble N	13.34	10.45	11.15	25.31	7.33	6.76	23.37	5.18	0.753	0.018	0.873	0.498	0.405	0.873

[1]Period 1 spanned 1 January 2018 – 16 February 2018; Period 2 spanned 9 April 2018 – 18 May 2018
[2]T = dietary treatment effect; P = period effect; T × P = dietary treatment and period interaction
[3]Contrasts: L = linear, Q = quadratic, C = cubic
[4]CO_2 = carbon dioxide, mg/g DM; CH_4 = methane, mg/g DM; N_2O = nitrous oxide, µg/g DM; Total CO_2e = CO_2 equivalent emissions (CH_4 + N_2O) , mg/g DM; Gross CO_2e = CO_2 equivalent emissions (CO_2 + CH_4 + N_2O) , mg/g DM; Emission factor = proportion of fecal nitrogen emitted as N_2O-N, µg/g
[5]Quebracho extract contained 28.9% protein precipitable phenolics
[6]SEM = standard error of the mean

Table A-21. Correlation coefficients for fecal nutrients and cumulative fecal gas fluxes

Items[1]	N	Soluble N	NDF	ADF	NFC	CO_2	CH_4	N_2O	Total CO_2e	Gross CO_2e
N	1.000[A]									
Soluble N	0.801*	1.000								
NDF	0.497*	0.059	1.000							
ADF	0.501*	0.066	0.961*	1.000						
NFC	0.385*	0.702*	-0.237*	-0.242*	1.000					
CO_2	-0.235*	-0.393*	0.219*	0.252*	-0.387*	1.000				
CH_4	-0.381*	-0.484*	-0.122	-0.086	-0.513*	0.466*	1.000			
N_2O	-0.093	-0.241*	0.103	0.092	-0.243*	0.339*	0.233*	1.000		
Total CO_2e	-0.378*	-0.499*	-0.099	-0.064	-0.525*	0.497*	0.987*	0.372*	1.000	
Gross CO_2e	-0.241*	-0.399*	0.214*	.248*	-0.394*	0.999*	0.483*	0.343*	0.514*	1.000

[A]Coefficient of correlation

* = $P \leq 0.05$

[1] N = nitrogen; NDF = neutral detergent fiber; ADF = acid detergent fiber; NFC = non-fibrous carbohydrates; CO_2 = carbon dioxide; CH_4 = methane; N_2O = nitrous oxide; Total CO_2e = CO_2 equivalent emissions (CH_4 + N_2O); Gross CO_2e = CO_2 equivalent emissions (CO_2 + CH_4 + N_2O)

Table A-22. Variance partitioning of covariance parameters for cumulative fecal gas fluxes

Gas[1]	Location[2]	Animal (T × P)[3]	Residual	*P*-value[4]
CO_2	CS	38.3%	61.7%	0.070
	SV	28.6%	71.4%	0.126
CH_4	CS	77.7%	22.3%	0.007
	SV	85.0%	15.0%	0.005
N_2O	CS	-	-	-
	SV	22.2%	77.8%	0.182
Total CO_2e	CS	67.1%	32.9%	0.013
	SV	81.3%	18.7%	0.006
Gross CO_2e	CS	39.3%	60.7%	0.066
	SV	28.5%	71.5%	0.127

[1]CO_2 = carbon dioxide; CH_4 = methane; N_2O = nitrous oxide; Total CO_2e = CO_2 equivalent emissions (CH_4 + N_2O); Gross CO_2e = CO_2 equivalent emissions (CO_2 + CH_4 + N_2O)
[2]CS = College Station; SV = Stephenville
[3]Animal (T × P) = animal nested within treatment and period
[4]*P*-value for Animal (T × P) using Wald Z-test

Table A-23. Ingredient and chemical composition of the high-roughage total-mixed ration utilized for metabolism and manure gas flux

	Basal diet
Items[1]	%
Ingredient composition, % DM	
Cottonseed hulls	37.00
Cracked corn	33.00
Alfalfa pellets	11.50
Bermudagrass hay	8.00
Molasses	7.00
Mineral	2.50
Urea	1.00
Chemical composition[2]	
DM, %	87.20
CP, % DM	12.90
Soluble protein, % CP	45.05
aNDF, % DM	48.70
ADF, % DM	35.85
Lignin, % DM	10.27
Crude fat, % DM	3.21
Sugar, % DM	3.30
Starch, % DM	20.30
NFC, % DM	32.00
Ash, % DM	5.86
Calcium	0.75
Phosphorus	0.43
TDN, %	60.95
NE_m, Mcal/kg	1.32
NE_g, Mcal/kg	0.76
GE, Mcal/kg[3]	3.85

[1]Items are feed ingredients and chemical composition of diets evaluated by Cumberland Valley Analytical Services (Waynesboro, PA).
[2]DM = dry matter; CP = crude protein; aNDF = neutral detergent fiber with amylase and sodium sulfate; ADF = acid detergent fiber; NFC = non-fiber carbohydrates; NE_m = net energy for maintenance; NE_g = net energy for gain
[3]GE measured by bomb calorimeter

Table A-24. Effect of quebracho extract on feed consumption and excretion profiles of steers fed high-roughage diets

	Quebracho extract, % of feed DM						Contrast[1] *P*-value		
Items[2]	0[3]	1.5	3	4.5	SEM[4]	*P*-value	L	Q	C
SBW, kg	238.14	235.87	235.08	235.02	2.19	0.465	0.159	0.484	0.914
Water intake, kg/d	8.15	9.45	7.92	7.21	1.37	0.440	0.324	0.311	0.407
Water intake, % SBW	3.25	3.87	3.25	3.00	0.60	0.530	0.481	0.319	0.406
Water intake:DMI	2.01	2.33	1.90	1.69	0.33	0.313	0.204	0.274	0.380
DMI, kg.d	4.01^{B}	4.03^{AB}	4.07^{AB}	4.13^{A}	0.03	0.036	0.005	0.522	0.933
OMI, kg/d	3.78^{B}	3.80^{AB}	3.84^{AB}	3.89^{A}	0.03	0.026	0.003	0.517	0.931
aNDFI, kg/d	2.34	2.31	2.31	2.31	0.02	0.464	0.159	0.484	0.920
ADFI, kg/d	1.55	1.54	1.53	1.53	0.01	0.518	0.177	0.561	0.861
Fecal DM, kg/d	1.49^{A}	1.61^{AB}	1.61^{AB}	1.77^{B}	0.06	0.003	<0.001	0.594	0.163
Fecal DM, % SBW	0.62^{A}	0.67^{AB}	0.68^{AB}	0.75^{B}	0.02	<0.001	<0.001	0.692	0.189
Fecal DM, %	27.55^{B}	28.79^{AB}	30.06^{A}	27.56^{B}	0.88	0.031	0.646	0.007	0.188
Fecal OM, kg/d	1.34^{A}	1.45^{AB}	1.43^{AB}	1.59^{B}	0.05	0.002	<0.001	0.513	0.073
Fecal aNDF, kg/d	0.99	1.03	1.00	1.09	0.04	0.186	0.079	0.450	0.271
Fecal ADF, kg/d	0.72	0.75	0.72	0.78	0.03	0.249	0.170	0.433	0.195
Urine, kg/d	5.36^{A}	5.58^{A}	4.26^{B}	4.24^{B}	0.33	<0.001	<0.001	0.602	0.015
Urine, % SBW	4.44^{A}	4.64^{A}	3.58^{B}	3.57^{B}	0.29	0.001	<0.001	0.597	0.022

[A-C]Least Squares means in a row with different superscripts differ at $P \leq 0.05$

[1]Polynomial Contrasts: L = linear, Q = quadratic, C = cubic

[2]SBW = shrunk body weight; DMI = dry matter intake; OMI = organic matter intake; aNDFI = neutral detergent fiber intake; ADFI = acid detergent fiber intake

[3]Quebracho extract contained 28.9% protein precipitable phenolics

[4]SEM = standard error of the mean

Table A-25. Effect of quebracho tannin percent on feed digestibility and N metabolism of steers fed high-roughage diets

	Quebracho extract, % of feed DM						Contrast[1] *P*-value		
Items[2]	0[3]	1.5	3	4.5	SEM[4]	*P*-value	L	Q	C
DMD, %	62.80^{A}	59.74^{A}	59.33^{AB}	55.72^{B}	1.39	<0.001	<0.001	0.782	0.201
OMD, %	64.60^{A}	61.39^{A}	61.58^{A}	57.64^{B}	1.40	0.001	<0.001	0.719	0.108
aNDFD, %	57.81	55.96	56.44	52.70	2.07	0.123	0.035	0.526	0.328
ADFD, %	53.43	51.60	52.76	48.83	2.21	0.204	0.087	0.508	0.260
N digestibility, %	48.66^{A}	39.77^{B}	41.10^{B}	35.49^{B}	2.24	<0.001	<0.001	0.314	0.026
Feed N, g/d	66.38	65.75	65.45	65.61	0.55	0.391	0.159	0.328	0.944
Fecal N, g/d	34.11^{A}	39.75^{B}	38.44^{B}	42.10^{B}	1.44	<0.001	<0.001	0.341	0.017
Fecal N, % N intake	51.34^{B}	60.23^{A}	58.90^{A}	64.51^{A}	2.24	<0.001	<0.001	0.314	0.026
Feces, % N	2.27^{B}	2.47^{A}	2.39^{AB}	2.38^{AB}	0.05	0.023	0.181	0.020	0.088
Urine N, g/d	21.23^{A}	17.42^{AB}	15.57^{AB}	13.44^{B}	2.04	0.007	<0.001	0.567	0.735
Urine N, % N intake	31.78^{A}	26.27^{AB}	23.59^{AB}	21.04^{B}	3.21	0.019	0.002	0.521	0.792
Urine, % N	0.41	0.35	0.38	0.35	0.03	0.300	0.175	0.476	0.248
Fecal N, % N excreted	62.01^{B}	69.57^{A}	71.81^{A}	75.69^{A}	2.45	<0.001	<0.001	0.299	0.378
Fecal N:Urinary N	1.68^{C}	2.36^{BC}	2.70^{AB}	3.24^{A}	0.30	<0.001	<0.001	0.749	0.564
Retained N, g/d	11.04	8.56	11.44	10.07	2.81	0.744	0.994	0.782	0.294
Retained N, % N intake	16.89	13.50	17.50	14.46	4.63	0.796	0.825	0.958	0.337

[A-C]Least Squares means in a row with different superscripts differ at $P \leq 0.05$

[1] Polynomial Contrasts: L = linear, Q = quadratic, C = cubic

[2]DMD = dry matter digestibility; OMD = organic matter digestibility; aNDFD = neutral detergent fiber digestibility; ADFD = acid detergent fiber digestibility; N = nitrogen

[3]Quebracho extract contained 28.9% protein precipitable phenolics

[4]SEM = standard error of the mean

Table A-26. Effect of quebracho tannin percent within a high-roughage diet on steer energy partitioning using open circuit, indirect calorimetry respiration chambers

	Quebracho extract, % of feed DM						Contrast[1] *P*-value		
Items[2]	0[3]	1.5	3	4.5	SEM[4]	*P*-value	L	Q	C
RQ	1.04^{A}	1.00^{B}	1.00^{B}	1.02^{AB}	0.009	0.001	0.079	<0.001	0.315
GEI, Mcal/d	15.82^{B}	15.96^{B}	16.19^{AB}	16.47^{A}	0.15	0.002	<0.001	0.497	0.936
GEI, kcal/MBW	260.86^{D}	264.96^{C}	269.41^{B}	274.24^{A}	0.62	<0.001	<0.001	0.420	0.990
FE, Mcal/d	5.99^{B}	6.57^{AB}	6.45^{B}	7.15^{A}	0.24	0.001	<0.001	0.711	0.058
FE, kcal/MBW	98.65^{B}	108.65^{AB}	107.53^{B}	118.96^{A}	3.84	<0.001	<0.001	0.796	0.067
DE, Mcal/d	9.82	9.38	9.74	9.32	0.26	0.180	0.185	0.953	0.075
DE, Mcal/kg DM	2.45^{A}	2.33^{AB}	2.38^{AB}	2.25^{B}	0.05	0.024	0.009	0.868	0.078
DE, % GE	62.20^{A}	59.05^{AB}	60.07^{AB}	56.63^{B}	1.44	0.009	0.003	0.890	0.075
UE, Mcal/d	0.27	0.24	0.22	0.24	0.03	0.491	0.284	0.292	0.735
UE, kcal/MBW	4.55	4.05	3.72	4.10	0.55	0.540	0.352	0.282	0.766
GASE, Mcal/d	1.04^{A}	0.98^{AB}	0.98^{AB}	0.87^{B}	0.04	0.007	0.001	0.430	0.269
GASE, kcal/MBW	17.17^{A}	16.22^{AB}	16.33^{AB}	14.50^{B}	0.81	0.029	0.006	0.454	0.261
GASE, % GE	6.56^{A}	6.10^{AB}	6.06^{AB}	5.30^{B}	0.32	0.008	0.001	0.517	0.272
ME, Mcal/d	8.49	8.14	8.53	8.20	0.29	0.450	0.599	0.961	0.132
ME, Mcal/kg DM	2.12	2.03	2.08	1.98	0.06	0.218	0.110	0.879	0.171
ME, % DE	86.57	86.90	87.57	87.96	0.91	0.435	0.110	0.958	0.831
ME, %GE	53.89	51.42	52.62	49.84	1.68	0.134	0.054	0.896	0.166
HE, Mcal/d	7.60^{A}	7.46^{AB}	7.23^{B}	7.30^{AB}	0.11	0.013	0.003	0.208	0.266
HE, kcal/kg MBW	125.19	123.79	120.42	121.54	1.76	0.058	0.019	0.325	0.262
HE, % GE	47.97^{A}	46.69^{A}	44.70^{B}	44.31^{B}	0.67	<0.001	<0.001	0.360	0.292
RE, Mcal/d	0.89	0.67	1.30	0.90	0.34	0.356	0.560	0.709	0.103
RE, Mcal/kg DM	0.23	0.18	0.31	0.22	0.08	0.482	0.737	0.680	0.149
RE, kcal/kg MBW	15.29	12.23	21.39	15.13	5.57	0.435	0.628	0.689	0.134
RE, %ME	10.50	7.96	14.70	10.78	3.75	0.375	0.530	0.798	0.110
RE, %GEI	5.92	4.73	7.92	5.52	2.08	0.483	0.762	0.686	0.147

[A-C]Least Squares means in a row with different superscripts differ at $P \leq 0.05$

[1] Polynomial Contrasts: L = linear, Q = quadratic, C = cubic

[2]RQ = respiratory quotient; GEI = gross energy intake; FE = fecal energy; DE = digestible energy; DM = dry matter; UE = urinary energy; GASE = gaseous energy; ME = metabolizable energy; HE = heat energy; RE = retained energy; MBW = metabolic body weight

[3]Quebracho extract contained 28.9% protein precipitable phenolics and 4.878 kcal/g of DM

[4]SEM = standard error of the mean

Table A-27. Variance partitioning of covariance parameters for metabolic and energy parameters of steers fed high-roughage diets

	Covariance parameter			*P*-value	
Items[1]	Animal	Period	Residual	Animal	Period
Metabolic parameters					
DMD, %	15%	30%	55%	0.176	0.159
OMD, %	11%	18%	72%	0.258	0.209
aNDFD, %	8%	14%	78%	0.302	0.237
ADFD, %	9%	8%	83%	0.298	0.304
N digestibility, %	6%	**60%**	34%	0.244	0.126
Fecal N, g/d	**45%**	33%	22%	0.048	0.129
Fecal N, % N intake	6%	**60%**	34%	0.245	0.127
Urine N, g/d	28%	-	72%	0.135	-
Urine N, % N intake	5%	-	95%	0.393	-
Fecal N, % N excreted	-	22%	78%	-	0.200
Fecal N:Urinary N	1%	16%	83%	0.476	0.231
Retained N, g/d	17%	17%	66%	0.185	0.206
Retained N, %N intake	10%	17%	73%	0.269	0.216
Energy parameters					
FE, Mcal/d	**59%**	20%	22%	0.044	0.142
DE, Mcal/d	**78%**	2%	28%	0.043	0.332
DE, Mcal/kg DM	10%	37%	53%	0.229	0.149
DE, % GE	11%	29%	60%	0.226	0.165
UE, Mcal/d	**40%**	4%	56%	0.084	0.335
GASE, Mcal/d	**66%**	13%	20%	0.041	0.151
GASE, % GE	**33%**	15%	52%	0.094	0.195
ME, Mcal/d	**55%**	5%	40%	0.057	0.282
ME, Mcal/kg DM	11%	34%	56%	0.221	0.155
ME, % DE	26%	16%	58%	0.120	0.199
ME, % GE	12%	28%	61%	0.220	0.169
HE, Mcal/d	**93%**	-	7%	0.033	-
HE, % GE	28%	7%	64%	0.122	0.283
RE, Mcal/d	16%	5%	79%	0.211	0.344
RE, Mcal/kg DM	24%	7%	69%	0.144	0.291
RE, % ME	24%	6%	70%	0.149	0.316
RE, % GEI	24%	7%	69%	0.147	0.300

[1]DMD = dry matter digestibility; OMD = organic matter digestibility; aNDFD = neutral detergent fiber digestibility; ADFD = acid detergent fiber digestibility; N = nitrogen; FE = fecal energy; DE = digestible energy; DM = dry matter; UE = urinary energy; GASE = gaseous energy; ME = metabolizable energy; HE = heat energy; RE = retained energy; GEI = gross energy intake;

Table A-28. Effect of quebracho tannin percent within a high-roughage diet on steer daily gas production, digestible nutrient per unit of gas produced, and retained nutrient per unit of carbon dioxide equivalent

	Quebracho extract, % of feed DM						Contrast[1] *P*-value		
Items[2]	0[3]	1.5	3	4.5	SEM[4]	*P*-value	L	Q	C
O_2, L/d	1508.41^{A}	1483.52^{AB}	1436.30^{B}	1453.30^{AB}	23.28	0.026	0.009	0.214	0.251
O_2, L/kg MBW	24.83	24.59	23.90	24.19	0.37	0.103	0.041	0.339	0.253
CO_2, L/d	1554.27^{A}	1514.09^{AB}	1469.24^{B}	1462.02^{B}	19.31	<0.001	<0.001	0.243	0.497
CO_2, L/kg MBW	25.57^{A}	25.07^{AB}	24.44^{B}	24.33^{B}	0.30	0.001	<0.001	0.366	0.513
CH_4, L/d	120.35^{A}	113.39^{AB}	112.79^{AB}	99.97^{B}	5.15	0.007	0.001	0.432	0.268
CO_2e, L/d	4924.14^{A}	4688.99^{AB}	4627.20^{AB}	4261.21^{B}	155.55	0.004	<0.001	0.559	0.344
CO_2e / OMI, L/g	1.29^{A}	1.21^{AB}	1.20^{AB}	1.09^{B}	0.04	0.004	<0.001	0.601	0.338
CO_2e / OMD, L/g	2.00	2.00	1.95	1.90	0.10	0.707	0.277	0.714	0.902
CO_2e / aNDFI, L/g	2.09^{A}	2.00^{AB}	2.00^{AB}	1.84^{B}	0.07	0.038	0.007	0.555	0.327
CO_2e / aNDFD, L/g	3.63	3.63	3.57	3.53	0.22	0.966	0.637	0.905	0.903
CO_2e / ADFI, L/g	3.15^{A}	3.01^{AB}	3.01^{AB}	2.78^{B}	0.11	0.038	0.007	0.549	0.328
CO_2e / ADFD, L/g	5.94	5.95	5.77	5.76	0.40	0.936	0.572	0.970	0.779
RE / CO_2e, cal/L	210.04	198.14	280.62	216.19	85.84	0.774	0.714	0.670	0.385
RN / CO_2e, mg/L	2.47	2.13	2.50	2.34	0.78	0.962	0.991	0.864	0.622

[A-C]Least Squares means in a row with different superscripts differ at $P \leq 0.05$

[1] Polynomial Contrasts: L = linear, Q = quadratic, C = cubic

[2] O_2 = oxygen, CO_2 = carbon dioxide; CH_4 = methane; CO_2e = carbon dioxide equivalent emissions (CO_2 + CH_4); OMI = organic matter intake; OMD = organic matter digested; aNDFI = neutral detergent fiber intake; aNDFD = neutral detergent fiber digested; ADFI = acid detergent fiber intake; ADFD = acid detergent fiber digested; RE = retained energy; RN = retained nitrogen, MBW = metabolic body weight

[3]Quebracho extract contained 28.9% protein precipitable phenolics

[4]SEM = standard error of the mean

APPENDIX B

FIGURES

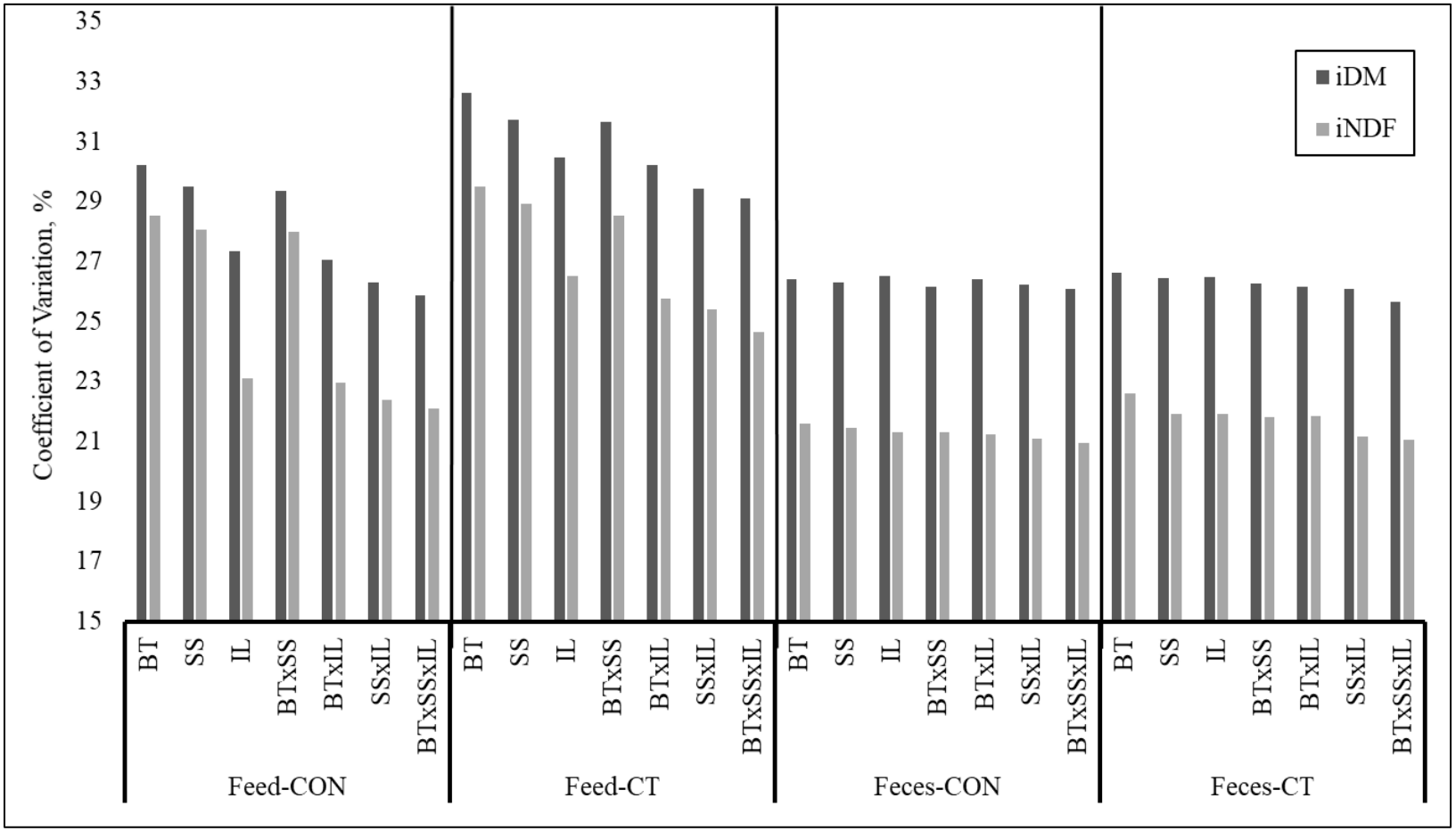

Figure B-1. Coefficients of variation for indigestible residues within sample types and diets. CON = control diet, CT = condensed tannin diet, BT = Bag type, SS = Sample size, IL = Incubation length, iDM = indigestible dry matter, iNDF = indigestible neutral detergent fiber.

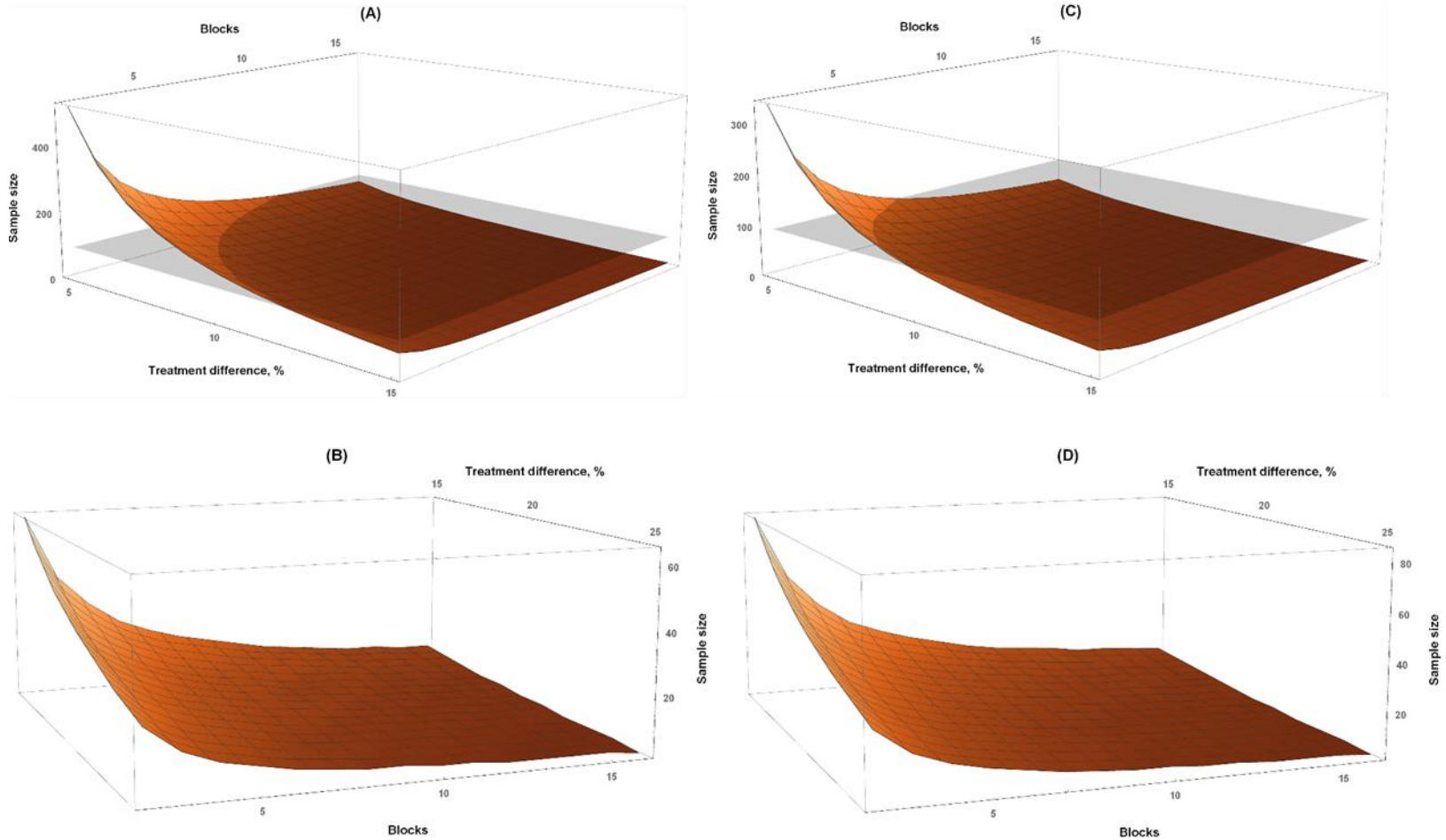

Figure B-2. Estimated sample size per treatment, number of blocks, and percent treatment difference required to detect a statistical difference among treatments for indigestible components (A = iDM feces, B = iDM feed, C = iNDF feces, D = iNDF feed), assuming a power of 90% and an α-value of 5%. The area below the shaded plane indicates differences capable of being detected with the sample size used in our study. The sample size and treatment differences for indigestible feed residues exceeded requirements for detection of differences within this simulation.

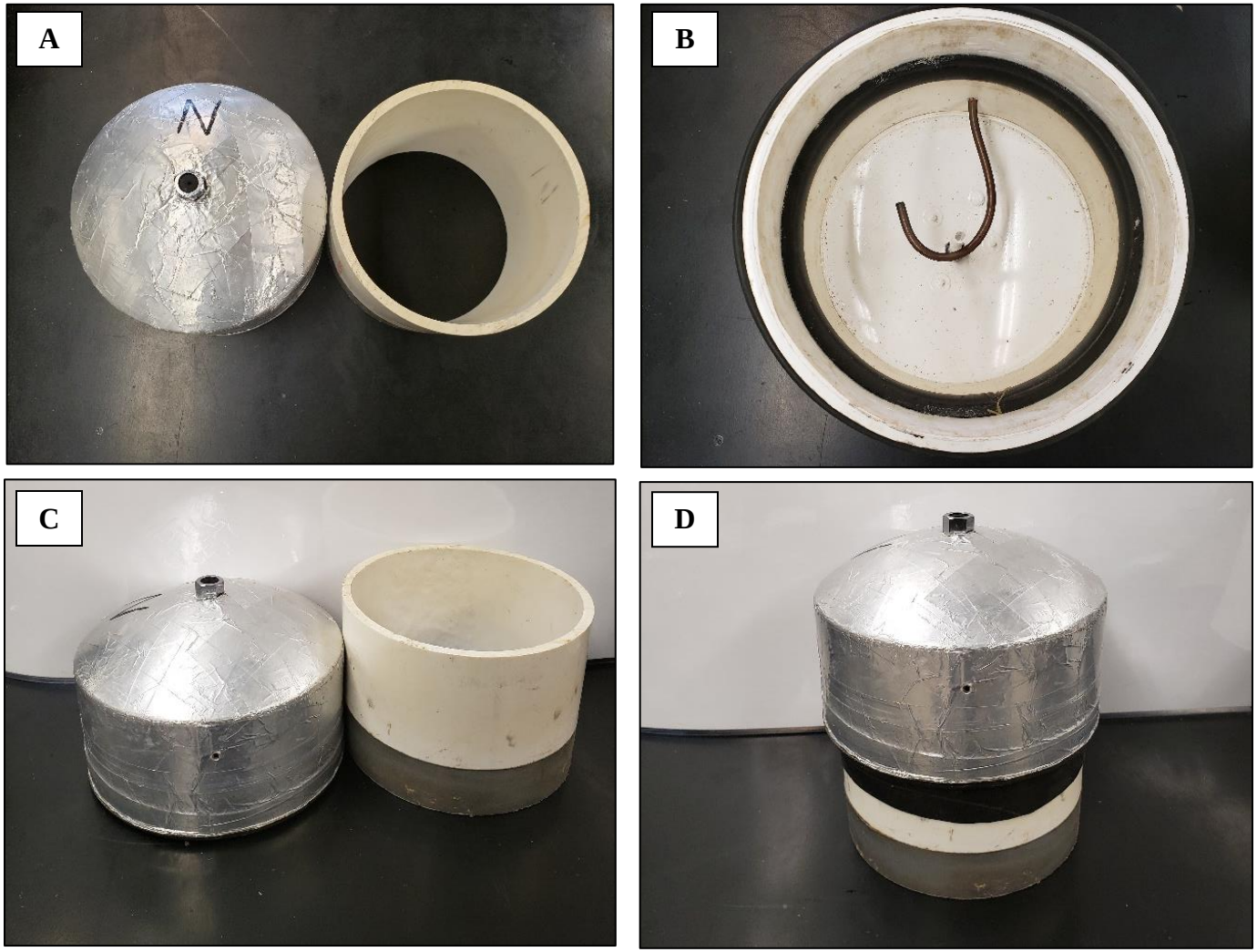

Figure B-3. Polyvinyl chloride collars and chamber caps used for the collection of fecal gases. A = Top view of chamber lid and collar; B = Inside of chamber lid; C = Side profile of chamber lid and collar; D = Chamber cap on top of chamber collar with rubber seal in place.

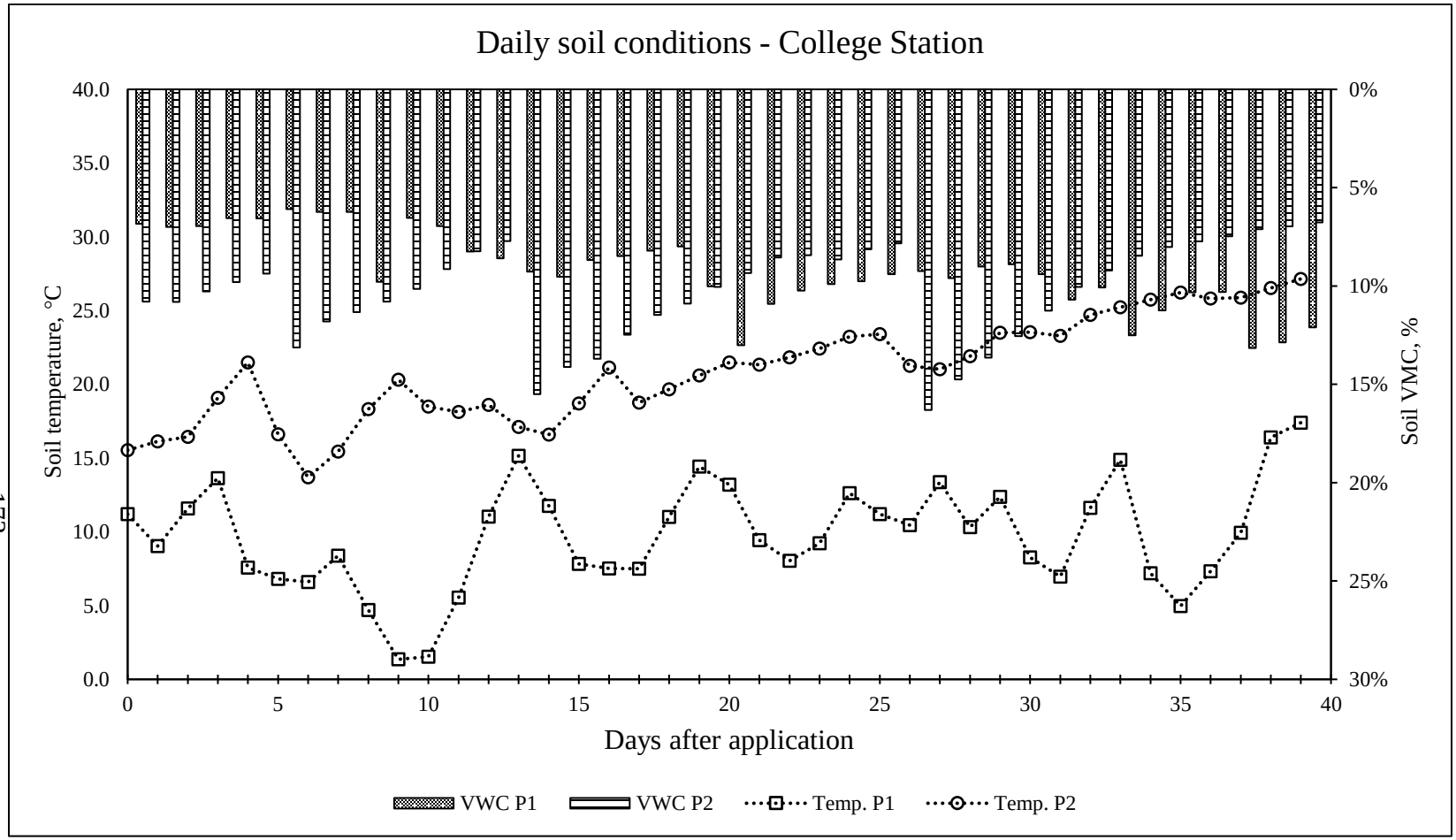

Figure B-4. Average daily soil conditions recorded between 0900 – 1100 h during two periods at the College Station location; P1 = period 1 (1 January 2018 – 16 February 2018), P2 = period 2 (9 April 2018 – 18 May 2018), VMC = volumetric water content.

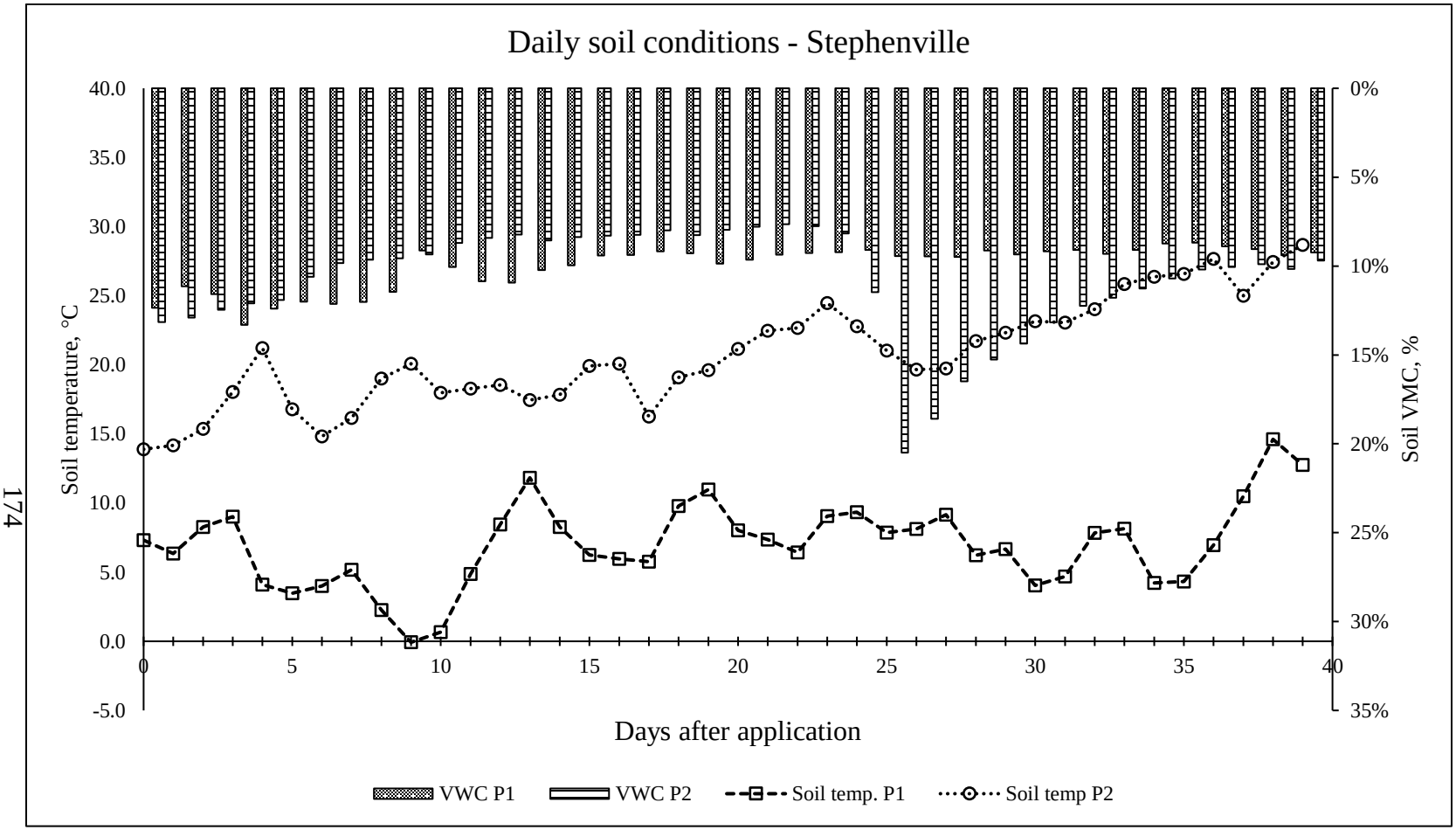

Figure B-5. Average daily soil conditions recorded between 0900 – 1100 h during two periods at the Stephenville location; P1 = period 1 (1 January 2018 – 16 February 2018), P2 = period 2 (9 April 2018 – 18 May 2018), VMC = volumetric water content.

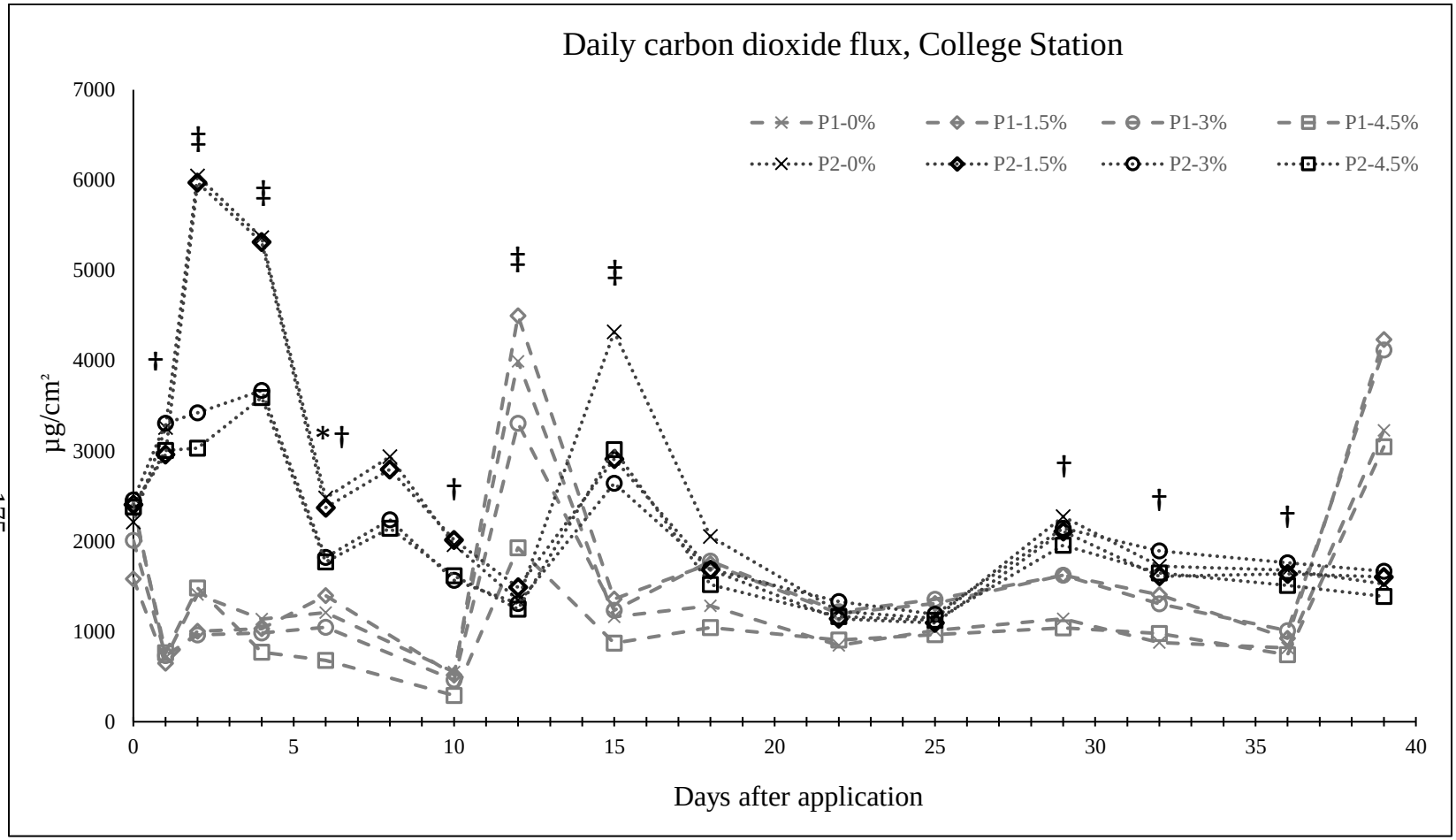

Figure B-6. Daily carbon dioxide flux during two periods at the College Station location; P1 = period 1 (winter), P2 = period 2 (spring); * = dietary treatment, † = period, and ‡ = dietary treatment × period interaction at $P \leq 0.05$ for individual days.

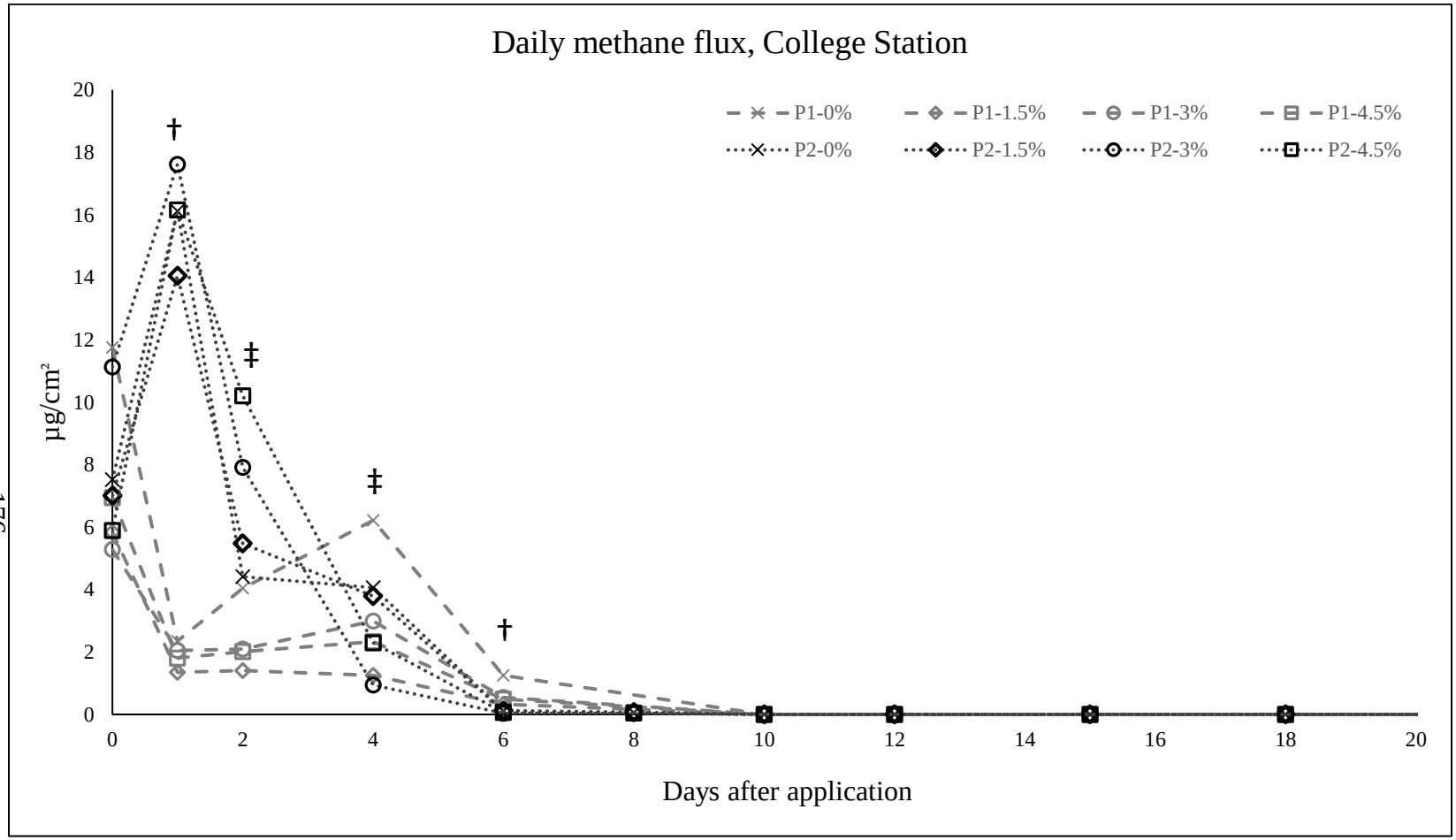

Figure B-7. Daily methane flux during two periods at the College Station location; P1 = period 1 (winter), P2 = period 2 (spring); † = period effect and ‡ = dietary treatment × period interaction at $P \leq 0.05$ for individual days.

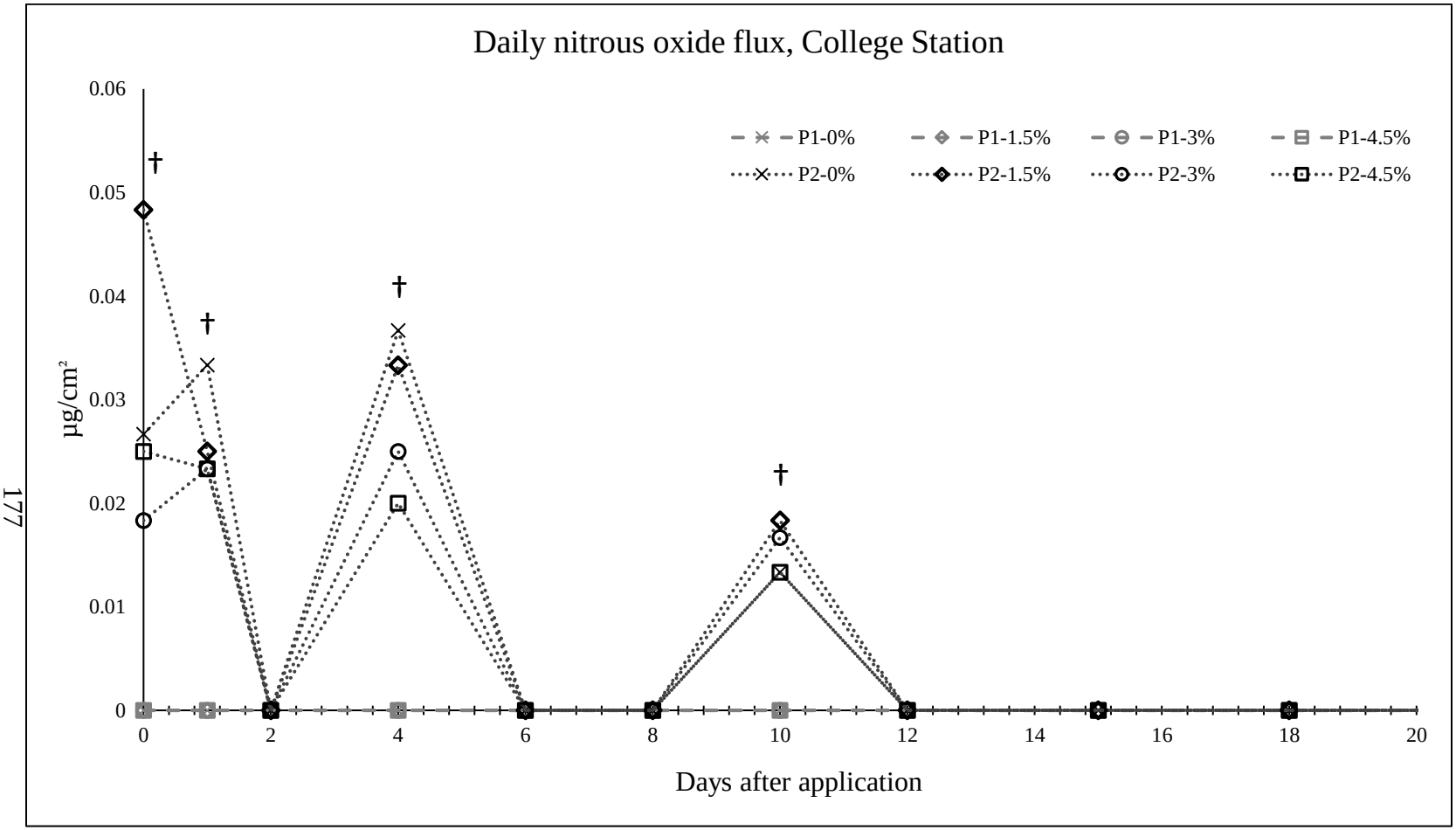

Figure B-8. The daily flux of nitrous oxide during two periods at the College Station location; P1 = period 1 (winter), P2 = period 2 (spring); † = period at $P \leq 0.05$ for individual days.

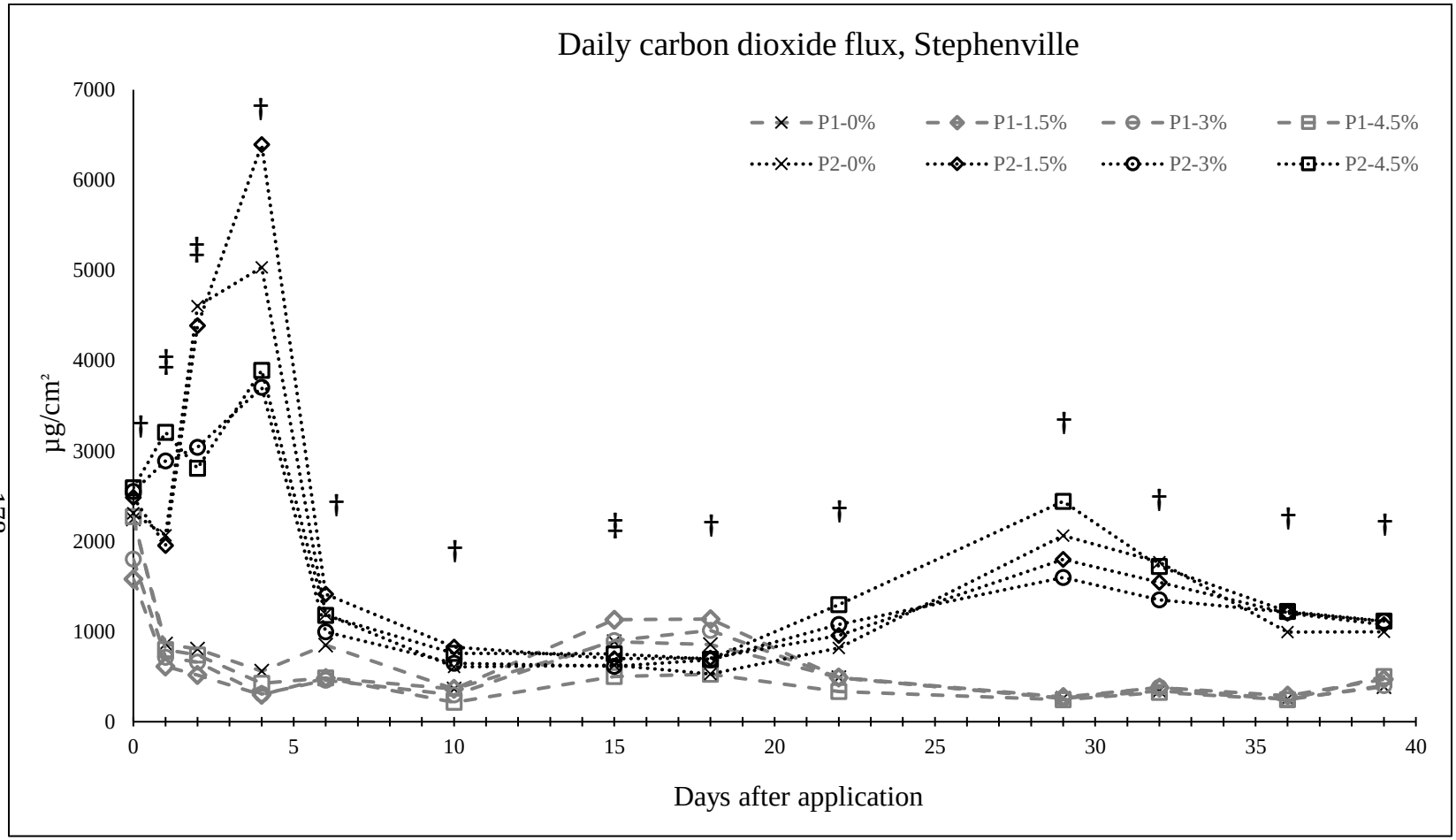

Figure B-9. The daily flux of carbon dioxide during two periods at the Stephenville location; P1 = period 1 (winter), P2 = period 2 (spring); † = period and ‡ = dietary treatment × period interaction at $P \leq 0.05$ for individual days.

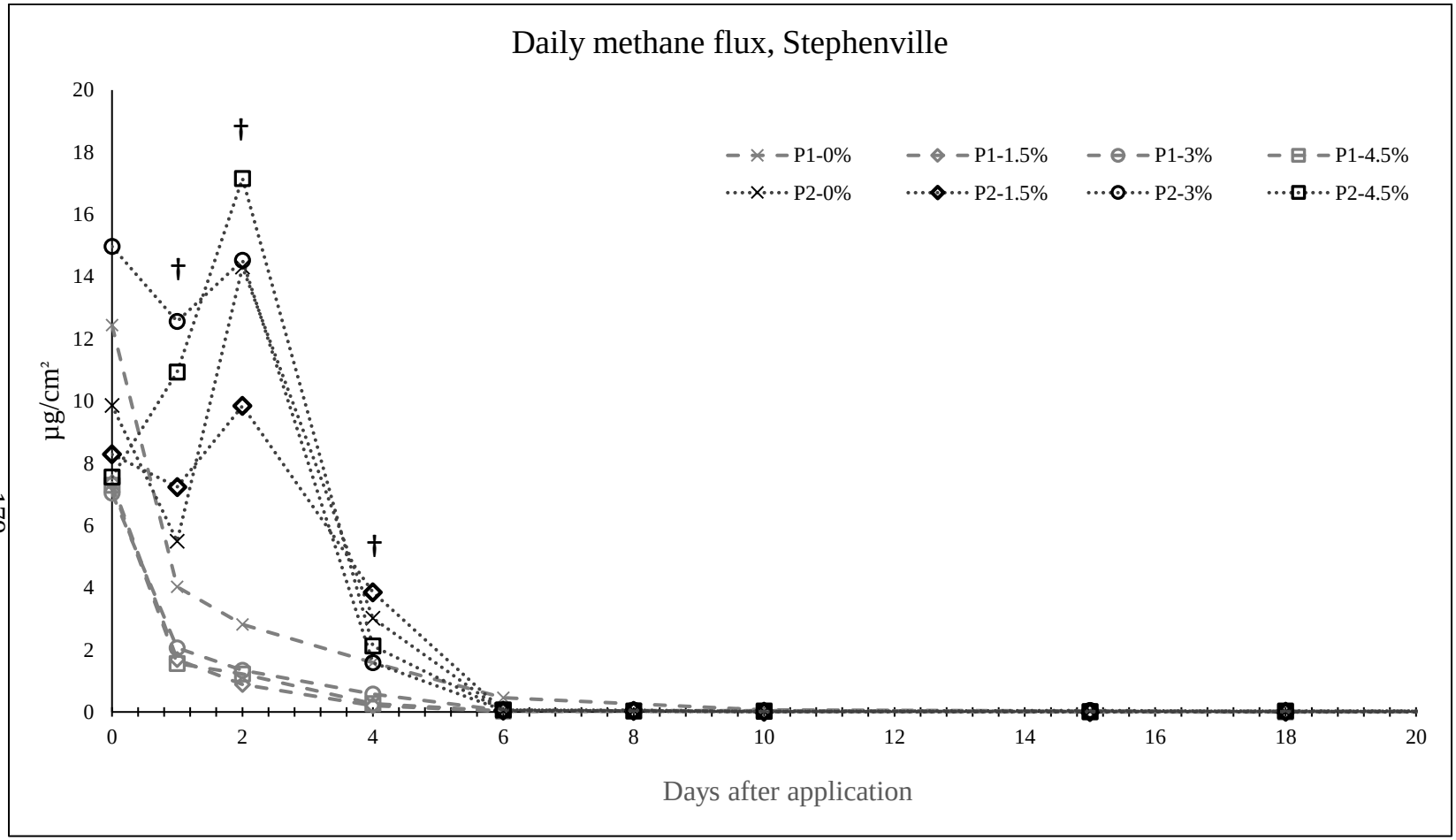

Figure B-10. The daily flux of methane during two periods at the Stephenville location; P1 = period 1 (winter), P2 = period 2 (spring); † = period at $P \leq 0.05$ for individual days.

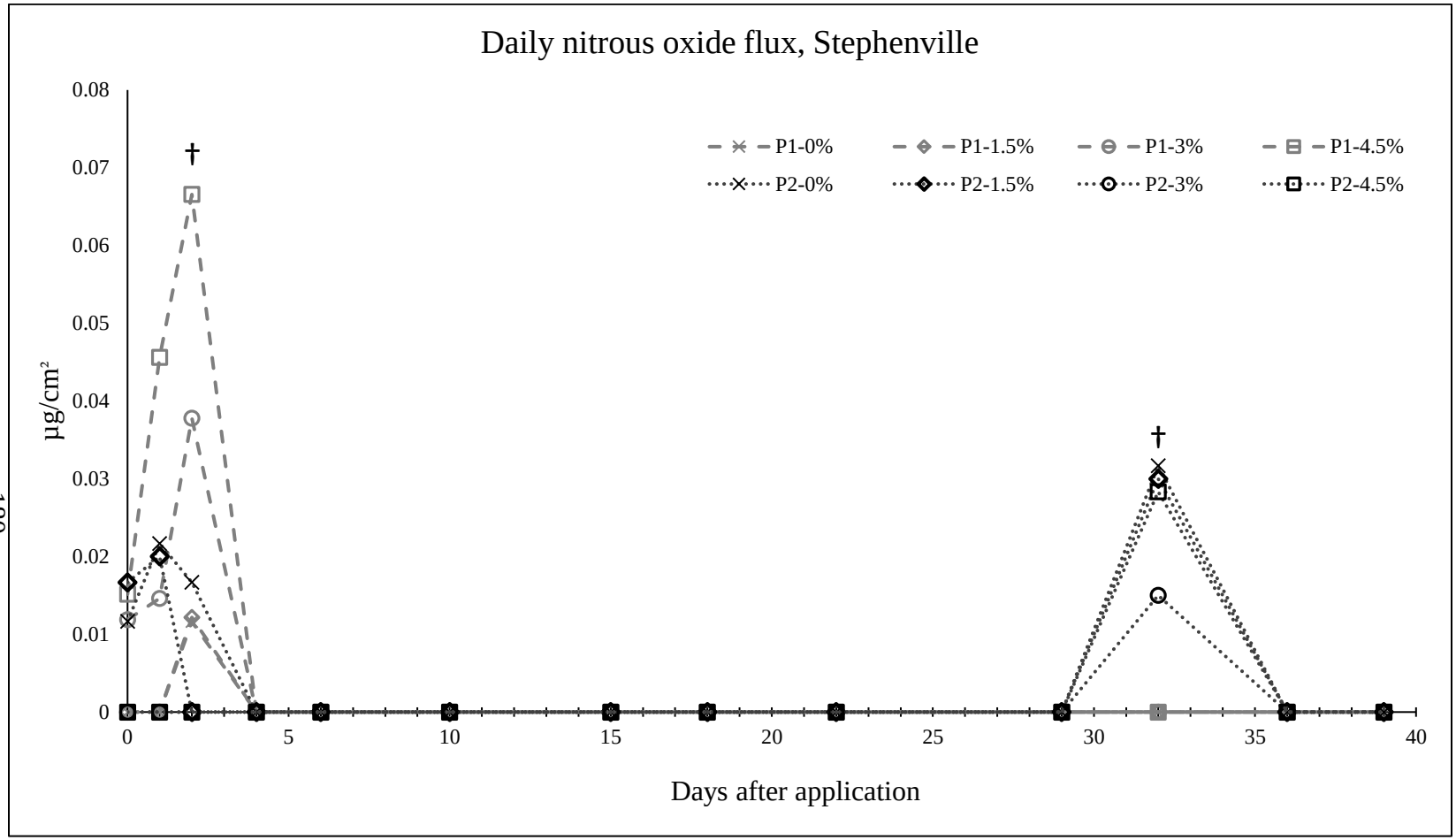

Figure B-11. The daily flux of nitrous oxide during two periods at the Stephenville location; P1 = period 1 (winter), P2 = period 2 spring); † = period at $P \leq 0.05$ for individual days